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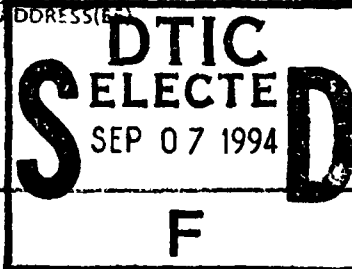
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The purpose of this grant was to investigate the metabolic and physiological factors determining the extent of the ischemic damage in a model of brief repetitive cerebral ischemia. The model is intended to simulate the effects of Gz induced blackout experienced by fighter pilots undergoing high gravitational stress maneuvers. To achieve this goal a rat model was developed whereby the ischemia was remotely induced by inflation (under computer control) of an occlusive cuff placed about the common carotid artery. Metabolic parameters were determined using *in vivo* NMR spectroscopy measurements throughout the ischemic and reflow periods. Lactate production was found to be highly reproducible and not limited by blood glucose levels. High energy phosphate decreases were correlated with loss of high frequency EEG. To evaluate the correlation between histologic damage and measures of regional metabolism we have carried out ¹H spectroscopic imaging experiments with histologic evaluations of the brain three hours after reperfusion. These studies were acquired with 5 minute (2 dimensional mapping across the cortex) and 16 sec time resolution (1 dimensional mapping) during the ischemia and reflow. Preliminary results of regional clearance kinetics of lactate shows a strong correlation with the presence of histologic damage.

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KA Conger, JH Halsey, KL Lou, MJ Tan, GM Pohost, HP Hetherington, Concomitant EEG, Lactate, and Phosphorous Changes by ^1H and ^{31}P NMR Spectroscopy During Repeated Brief Cerebral Ischemia, , Journal of Cerebral Blood Flow and Metabolism, 1994 in press.

Abstracts National Meetings:

KA Conger, HP Hetherington, MJ Tan, KL Lou, GM Pohost, JH Halsey, Simulating Lactate Accumulation During Brief Repetitive Cerebral Ischemia, Proceedings of the Society of Magnetic Resonance in Medicine, #2158, Berlin Germany, 1992.

KA Conger, JH Halsey, MJ Tan, KL Lou, GM Pohost, HP Hetherington, Concomitant EEG, Lactate, and Phosphorous Changes During Repeated Brief Cerebral Ischemia, Proceedings of the Society of Magnetic Resonance in Medicine, New York New York 1993.

B. Books or Book Chapters Published

None.

C. Graduate Students

None.

D. Post Doctorates

Tan, Min-Jie, Neurology non-US

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I INTRODUCTION

The purpose of this grant was to investigate the metabolic and physiological factors determining the extent of the ischemic damage in a model of brief repetitive cerebral ischemia. The model is intended to simulate the effects of Gz induced blackout experienced by fighter pilots undergoing high gravitational stress maneuvers. To achieve this goal a rat model was developed whereby the ischemia was remotely induced by inflation (under computer control) of an occlusive cuff placed about the common carotid artery. Metabolic parameters were determined using in vivo NMR spectroscopy measurements throughout the ischemic and reflow periods. The time courses of lactate production and high energy phosphate changes have been correlated with EEG changes and are described in the two attached manuscripts. To evaluate the correlation between histologic damage and measures of regional metabolism we have carried out ¹H spectroscopic imaging experiments (mapping the lactate changes spatially across the brain) with histologic evaluations of the brain three hours after reperfusion. These studies were acquired with 5 minute (complete 2 dimensional mapping across the cortex) and 16 sec time resolution (1 dimensional mapping acquiring 16 columns across the brain from right to left) during the ischemia and reflow (3 hours) periods. At this time analysis of all of the NMR and histologic data has not been completed, however results from five of the animals indicates that the regional clearance kinetics of lactate are strongly correlated with the presence of histologic damage.

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II. PROGRESS

A. Time Course of Metabolite Changes during Repetitive Ischemia

1.Introduction: The goal of this work was to evaluate the temporal progression of the metabolic and functional changes accompanying repetitive brief cerebral ischemic episodes. To evaluate the time course of the metabolic changes serial interleaved ^1H and ^{31}P NMR spectra were collected while rats were subjected to 30 one minute episodes of cerebral ischemia and reflow followed by thirty minutes of continuous reflow. To evaluate the effect of the repeated ischemic episodes on brain function, fast and slow components of the EEG were monitored throughout the ischemic and reflow periods. The severity of the ischemia was modulated by altering the ischemic duration during the one minute period.

2.Methods: Male Sprague Dawley rats (200-250 gm) were anesthetized with 70% N_2O , 29% O_2 , and 1% Halothane, paralyzed with tubocurarine chloride and mechanically ventilated. The external carotid and both subclavian arteries were ligated. The right common carotid artery was bi-directionally cannulated for monitoring the carotid stump pressure (reflecting the cerebral perfusion pressure at the Circle of Willis) and for measurement of systemic pressure. Rectal temperature was monitored throughout the experiment and maintained at 37°C by use of feedback regulated heating chamber surrounding the animal. Ischemia was induced by an inflatable occlusive cuff placed about the left common carotid artery. Inflation and deflation of the cuff was controlled by a hydraulic system linked to a IBM AT computer. EEG activity was monitored by placement of two silver wire electrodes. The resultant signal was analog filtered to obtain fast (7-20Hz) and slow (2-5Hz) components and digitized every half second throughout the experiment.

Each experiment consisted of: 1) an initial period of 10 minutes for control measurements; 2) followed by 30 minutes of 30 episodes of brief cerebral ischemia and reflow; 3) concluding with 30 minutes of continuous reflow. The severity of the ischemia was modulated by altering the durations of the ischemic and non-ischemic durations during the one minute interval. Four protocols were utilized in 20 studies: 10I:50R, 10 seconds of ischemia followed by 50 seconds of reflow (n=6), 20I:40R (n=6), 30I:30R (n=4) and 40I:20R (n=4).

NMR data was collected using a 4.7T 40cm bore Bruker Biospec system using a two coil $^1\text{H}/^{31}\text{P}$ detector. An elliptical ^{31}P surface coil (8x12mm) was placed directly upon the exposed skull. The ^1H coil, was placed immediately above the ^{31}P coil and consisted of two 10mm circular loops connected in a butterfly configuration. The butterfly configuration minimizes coupling between the coils. To minimize the acquired lipid signal, the scalp was removed and the temporal muscles retracted. ^1H and ^{31}P spectra were acquired simultaneously by interleaving ^1H and ^{31}P acquisitions on a scan by scan basis. The data was acquired in one minute bins, (60 scans at a 1 second repetition time for both nuclei). ^{31}P spectra were summed in a moving average of four adjacent one minute measurements to improve the S/N. Resonance areas were determined by

peak integration. ^1H resonance areas were converted to millimolar values using a 11mM value for cerebral creatine as an internal standard, and corrected for NMR measurement parameters.

3. Results:

i. Ischemia Model: The computer controlled occluder system provided a highly reproducible ischemic insult with minimal occlusion to occlusion variations in the carotid stump pressure during each subsequent ischemic episode. The carotid stump pressure during the ischemic period was typically 7-10mM with variations of approximately 1-2mM seen for a given animal.

ii. EEG Activity: Both the slow and fast components were seen to decline during the ischemic interval of each ischemia/reflow episode in the 20I:40R, 30I:30R and 40I:20R animals. Minimal changes were seen in the 10I:50R animals, most likely due to the shortness of the interval and the finite time required for cuff inflation and consumption of brain oxygen stores. To facilitate the analysis of long term trends, the EEG data was averaged over the one minute ischemia/reflow episode and for all animals in each group. In the 10I:50R animals the average fast activity was unchanged and a mild increase in slow activity was seen. Fast and slow activity was seen to decline in the 20I:40R, 30I:30R, and 40I:20R protocols achieving an average decline of greater than 80% in fast activity in the 40I:20R protocol. The 20I:40R animals showed an initial rapid decline in EEG until reaching an apparent steady state at approximately 60% of control period fast activity. Slow activity in these animals was also found to decline but a mild recovery was subsequently seen. In the 30I:30R and 40I:20R animals, the decline in average EEG activity was found to be progressive with the depth of the depression in each period increasing with each successive ischemic episode. This trend continued until the EEG was virtually gone in the 40I:20R animals. Upon reflow all groups showed a recovery of fast and slow activity reaching levels near or above control values by the end of thirty minute reflow period.

Therefore, the 10I:50R protocol appears to establish a threshold for ischemic duration in this model below which significant changes in fast EEG activity do not occur. This minimum interval most likely reflects the amount of time required to establish the ischemia (several seconds for balloon inflation) and deplete brain oxygen stores (6). The 20I:40R animals establish an apparent steady state of decreased EEG, such that the effects of each ischemic episode on EEG are balanced by each reflow period. In contrast, the 30I:30R and 40I:20R animals show an incremental decline throughout the thirty episodes of ischemia in EEG activity reflecting the inability of the brain to compensate for the cumulative effects of each new ischemic period. Specifically, the amount of fast EEG activity during each reflow period was found to decline. Thus the 30I:30R animals set a lower threshold for a slow progressive decline in EEG activity.

iii. ATP, PCr and Pi: Similar to the EEG, the 10:50 ischemic protocol also did not result in a significant decrease in PCR when the ^{31}P data was averaged over 4 consecutive ischemia/reflow episodes. However significant declines in PCR content

relative to the control period were observed in the other groups. As expected the longer ischemic intervals resulted in greater decline in PCr, with the 40I:20R protocol resulting in a 75% decrease in PCr. The PCr time course data is qualitatively similar to that of the EEG. The 20I:40R animals showed a rapid decline followed by a plateau region (75% of control) whereas the 30I:30R and 40I:20R animals showed rapid decreases followed by slow progressive declines. All animals showed near complete PCr recovery at the end of the thirty minutes of continuous reflow.

Significant changes in average ATP were visualized only in the 40I:20R animals. This is consistent with a model where ATP is preserved at the expense of PCr. Therefore the 50% average decrease in PCr seen in the 30I:30R animals does not appear to be sufficient to induce a significant change in the average ATP content, despite substantial changes in the EEG. This effect may reflect a strong link between PCr and EEG activity or possibly reflect the averaging nature of the acquisition. Specifically, short term reductions in ATP during each ischemic interval may occur which might not appear as significant decreases in the 4 minute ischemia/reflow average data.

Cerebral P_i levels are reported as a fraction of the pre ischemia PCr content. Similar to that seen in the ATP data a significant increase in P_i content is visualized only in the 40I:20R animals, where substantial ATP hydrolysis augments the PCr dephosphorylation. However, the change in the 40I:20R group is substantial, displaying an increase of greater than 3 fold.

iv. Lactate and pH: The resonance at 1.33 ppm has contributions from extracerebral lipids, lactate, threonine and alanine and lactate. To minimize the effects of these contaminants, the lactate data is reported as a difference in the 1.33 ppm resonance area from the average control value. Since contributions from extracerebral lipids should not be changing during the experiment and changes in lactate in millimolar quantities should dominate the changes in alanine and threonine, the difference data should provide a good estimate of the lactate accumulation induced by the ischemia. In this study lactate proved to be the most sensitive indicator of ischemia, showing significant elevations in all four groups. Similar to the changes in EEG, lactate levels in the 20I:40R group showed a rapid increase to a plateau level. The plateau level resulting from a complete balance between clearance and generation rates. Lactate changes in 30I:30R and 40I:20R groups again were qualitatively similar to the EEG changes showing rapid initial elevations followed by slow progressive increase. Despite the large changes in lactate observed in 40I:20R animals, approximately 18mM, lactate clearance during the subsequent reflow period was complete, with all animals returning to near control levels.

The time course of pH changes were qualitatively similar to that seen in the lactate data. The observed pH changes ranged from initial levels of 7.15 in the control period to 7.1 at the conclusion of the ischemic episodes in the 10I:50R group to 7.0, 6.8 and 6.6 in the 20I:40R, 30I:30R and 40I:20R animals respectively. Again all animals showed complete recovery to control values during the reflow period. Correlation of the

pH and lactate data revealed a general trend towards decreasing pH with increasing lactate. However, the data was found to segregate according to duration of ischemia with decreased pH being associated with increased ischemic duration despite identical lactate levels.

vi. EEG - Metabolic Correlations: Significant changes in lactate, pH and PCr content were found in all animals that approximately paralleled the decreases in EEG activity. To evaluate the relationship between these metabolic (lactate, pH and PCr) and functional variables (fast and slow components of the EEG), EEG was plotted as a function of lactate, pH and PCr. Although all three variables showed strong trends with fast activity, the relationship between lactate activity was different depending on the ischemic group viewed. Specifically, greater declines in fast activity were observed for the same increase in lactate in the longer duration ischemias. Excellent correlations were seen between the pH and PCr levels in the 20I:40R, 30I:30R and 40I:20R animals.

4. Summary: We have demonstrated that brief repetitive ischemias can produce progressive highly reproducible declines in PCr, pH and increases in lactate. The severity of these changes is easily modulated by altering the ratio of the ischemic to reflow period during each episode. EEG was also shown to decline in this model and be most strongly correlated with changes in PCr and pH. Lactate changes were found to parallel the changes in EEG, however the level of lactate accumulation was found to vary substantially for the same decrease in EEG. Specifically, larger declines in average EEG were seen at equivalent lactate levels for animals subjected to longer ischemic periods. The PCr and EEG data are consistent with a model whereby the amount of fast activity is directly proportional to the availability of PCr. The strong relationship between pH and EEG is most likely influenced by the creatine kinase equilibrium, and therefore at least partially reflects the availability of PCr. Finally, the ischemia appears to be fully reversible, since all metabolic and functional correlates returned to control levels. This complete metabolic recovery may permit each animal to serve as its own control in determining the metabolic effect of different therapeutic interventions. However, histologic evaluations were not performed on these animals, such that the presence of cellular damage can not be excluded in the more severe protocols. Studies investigating differences in regional metabolic sensitivity and histologic correlations are currently underway.

B. Substrate Utilization and Lactate Clearance Rates

1. Introduction: Previously at relatively low time resolution (1 minute) we have demonstrated that lactate production during 30 consecutive periods of reflow and ischemia follows an approximately exponential increase to a plateau level (section A). To evaluate the determinants of lactate production that give rise to this accumulation profile, we have made rapid serial measurements of lactate (5 or 10 second time resolution) by in vivo ^1H NMR spectroscopy. We have used these measurements to 1)

quantitate the ischemic production and clearance rates and 2) use these measured values to model lactate accumulation during thirty sequential periods of ischemia and reflow.

2. Methods: Seven male Sprague Dawley rats (200-240gm) were prepared surgically as described in section II.A.2. A brief (60 second) sustained occlusion followed by 15 minutes of reflow was used to measure the rate of lactate production during ischemia. The repetitive ischemia protocol consisted of 30 consecutive 30 second (nominal duration) ischemia and 30 second reflow periods followed by 30 minutes of continuous reflow. The one minute occlusion protocol was carried out before the repetitive ischemic protocol.

NMR data was collected using a 4.7T 40cm bore Bruker Biospec system using a single tuned 8x12mm elliptical ^1H surface coil placed directly on the skull. To minimize the acquired lipid signal, the scalp was removed and the temporal muscles retracted. During the sixty second sustained ischemic periods, spectra were acquired with 5 second time resolution ($\text{TR} = 1250$ msec, 4 averages). During the repetitive ischemia protocol and the first ten minutes of continuous reflow, spectra were acquired with 10 second time resolution ($\text{TR}=1250$ msec, 8 averages). During the final 20 minutes of reflow, spectra were acquired every 30 sec ($\text{TR}=1250$, 24 scans). Resonance areas were determined by peak integration. Resonance areas were converted to millimolar values using a 11mM value for cortical creatine, and NMR determined values.

Lactate accumulation during the repetitive ischemia period was modeled as the balance between lactate production at a constant rate ($d[\text{lactate}]/dt = k_p$) and a first order clearance process ($d[\text{lactate}]/dt = -k_c[\text{lactate}]$). The value of k_p for each animal was determined from a one minute continuous occlusion before the repetitive ischemia protocol. The value of k_c was determined from the reflow period following thirty cycles of the repetitive ischemia protocol. The exact duration of the ischemic period in each animal was determined from the carotid pressure recordings. Due to the small residual flow experienced during the ischemic period $12\text{ml}\cdot 100\text{g}^{-1}\cdot \text{min}^{-1}$ lactate clearance was assumed to occur continuously throughout the ischemic and reflow intervals. The calculated values for k_p and k_c along with the ischemia duration for each animal were then used to predict the time course of lactate accumulation during the repetitive ischemia protocol.

3. Results:

i. Lactate Clearance Kinetics: The rate of lactate clearance was determined in each animal using the equation

$$[\text{lactate}](t) = [\text{lactate}]_0 \exp(-k_c t)$$

where $[\text{lactate}]_0$ is the lactate present at the beginning of the thirty minute reflow period and t is the time in minutes, and k_c is in units of min^{-1} . The lactate level is reported as a difference from the control period to minimize the effect of resonance overlap with other metabolites and lipids. The measured clearance rates are reported in table 1 along with the mean and standard deviations ($0.143 \pm 0.032 \text{ mM}\cdot \text{min}^{-1}$) for all seven

animals. This value is in good agreement with the lactate transport rate of $0.150 \text{ } \mu\text{mole} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ reported by Drewes and Gilboe in the perfused dog brain (7).

ii. Lactate Generation Kinetics: The lactate production rate was determined from a one minute continuous occlusion in each animal prior to the repetitive ischemia protocol. The lactate difference data was fit using a linear production rate and a correction for lactate clearance

$$[\text{lactate}](t) = k_p t - k_c t [\text{lactate}]$$

where k_p the production rate in $\text{mM} \cdot \text{min}^{-1}$. The value of k_c used was determined as previously described. Calculated values for k_p for all seven animals are listed in table 1. The values reported here ($4.63 \pm 0.73 \text{ mM} \cdot \text{min}^{-1}$) are somewhat lower than those reported by Nilsson(8), $7.0 \text{ } \mu\text{moles} \cdot \text{gm} \cdot \text{min}^{-1}$. This may reflect differences in the glycolytic rate arising from anesthesia and or the small residual flow experienced in the repetitive ischemic model (typical CSP = 7-10mm Hg).

iii. Determination of Ischemic Duration: Due to the finite time for inflation and deflation of the occlusive cuff, the actual ischemic periods were found to be shorter than 30 seconds and vary slightly from animal to animal. Additionally, substantial lactate generation or anaerobic glycolysis will only occur after brain oxygen stores have been depleted. Lowry (6) has reported that lactate generation begins after a 3 second time delay in animals subjected to complete global ischemia. To account for the time delay in inflating the occluder and the subsequent delay to consume brain oxygen stores, the duration of the ischemic period was defined as the time during which the carotid pressure was within 1mm of the ischemic minimum value less three seconds. This correction shortened the mean ischemic duration to $21 \pm 1 \text{ sec}$ and the individual times for each animal are reported in table 1.

iv. Lactate Accumulation during Repetitive Ischemia: The time course of lactate accumulation during the 30 consecutive ischemia/reflow periods was modeled using the rate constants, k_p and k_c and the ischemic duration calculated from the carotid pressure measurements. The incremental change in lactate during each ischemic and reflow period was calculated using the lactate generation rate (k_p) corrected for the duration of the ischemic period ($t_{\text{isch}}/60$) minus clearance during the entire interval ($k_c [\text{lactate}]$).

$$[\text{lactate}] = k_p (t_{\text{isch}}/60) - k_c [\text{lactate}]$$

To evaluate the accuracy of the calculated time courses, the observed data, calculated data and calculated data assuming 15% errors in the production rate constants. All animals showed excellent agreement, with nearly all measured values lying within the 15% error bounds. Assuming error bounds of 15% in the clearance rate provides similar limits.

The high sensitivity of the shape and absolute level of lactate accumulation to clearance rate, production rate and ischemic duration and the agreement of the model and observed data suggest that both the model used and the rates measured are highly accurate, (within 15%). The assumption that lactate clearance occurs throughout the

"ischemic" period in this model is also supported, since a 33% decrease in reflow time would cause large changes, in the total amount of lactate produced.

4. Summary: We have demonstrated that the accumulation of lactate during a brief repetitive ischemia protocol can be accurately modeled (<15% error) using rate constants determined on an individual animal basis. Thus the simple model of lactate production at a constant rate during each ischemic period, and washout throughout the reflow and ischemic period provides an excellent model for predicting the amount of lactate accumulated during the protocol. The observed lactate production during each ischemic period appears to occur at the same rate as during a continuous one minute ischemic episode and these rates are consistent with the maximum glycolytic rates observed by Nilsson (8) in a model of complete global ischemia. Thus, lactate production in this repetitive ischemia model does not appear to be limited by glucose or other substrate availability. This indicates that glucose replenishment during the reflow period is sufficient to support the elevated level of anaerobic activity. This is in contrast to continuous global ischemia models where the amount of lactate produced is dependent on the brain glucose and glycogen stores immediately prior to the induction of ischemia. Thus glucose availability is unlikely to be a major factor in determining the extent of ischemic damage in this model. However, if lactate accumulations are deleterious, via pH and or osmotic changes, then interventions to alter the efficiency of the monocarboxylic acid transporter may provide a mechanism for tissue preservation. The correlation of lactate accumulation and histologically visualized damage forms the next area of our research.

C. Correlation of Ischemic Damage and Regional Lactate.

1. Introduction: As described, our previous studies focused on the issues of metabolite and functional alterations during the ischemic protocols from the entire brain. However, they have not addressed the issue of focal tissue damage and selective vulnerability. To pursue this question we have implemented a ^1H spectroscopic imaging sequence to evaluate the regional time course of lactate elevation. This method permits us to acquire data from 256 individual spatial locations (approximately one half within the brain) simultaneously during a five minute measurement. The volume elements investigated here are approximately $1 \times 1 \times 4 \text{ mm}$. This method has been used to assess the regional time courses of lactate elevation in a 50:20 ischemia reflow protocol, 30 occlusions, followed by thirty minutes of reflow. A three hour reflow time was chosen to facilitate the histologic evaluation of the damage.

2. Methods: Animals were prepared as described previously in section A.1 with the exception that the carotid stump pressure was not monitored allowing both the right and left common carotids to be occluded remotely. This eliminated the possibility that a preferential sidedness due to the permanent occlusion of one carotid would be seen. Data was acquired from three animal groups. One animal group (5 rats) served as a sham group, undergoing the complete protocol but not experiencing the ischemic period. A second group (10 rats) were subjected to thirty five minutes of 50:20 ischemia followed by three hours of reperfusion and fixation. Full 2-dimensional ^1H spectroscopic

images (16x16 voxels across the brain) were acquired with five minute time resolution throughout the ischemic interval and the first hour of reflow. During the final two hours of reflow data was acquired every 15 minutes. The final group underwent the identical protocol however 1 dimensional data was acquired every 20 seconds (16 columns across the head to provide better lactate generation kinetics).

Rat brains were perfused in situ through the carotid artery, beginning with a normal saline rinse (30 seconds)(11) containing 10 units per milliliter of heparin, 0.1% procaine HCl, pH 7.4 and a final osmolality of 380 mOsM followed by perfusion with fixative solution containing 2% formaldehyde, 1% glutaraldehyde in 0.15 M Na phosphate, pH 7.4 (1000 mOsM). Following perfusion fixation the rats were decapitated and their heads placed in 10% buffered formalin overnight at 4°C. This delay prevents generation of artifactual "dark neurons" (11). The brains were then removed from the skull.

Standardized serial coronal sections were cut at one mm intervals throughout the brain using an Oxford Vibratome and subsequently scanned with a 12 bit Molecular Dynamics Personal Densitometer. The scans were then processed with Molecular Dynamic's ImageQuant™ software to provide pseudo colored images proportional to measured absorbances (see figure 1). Damaged areas in tissue stand out as higher absorbance areas compared to normal brain (figure 1). Preliminary results indicate that pseudocolored can adequately distinguish marked differences in tissue absorbance which correlate well with observed slower rates of lactate clearance detected by ¹H NMRS imaging of lactate.

One micron sections of plastic embedded tissue prepared from epoxy embedded brain tissue will be used for this study. Although, we had originally expected to embed in glycolmethacrylate to perform the neuronal evaluation, we have decided to modify this approach and embed in Spur media which will permit both neuronal evaluation in one micron "thick" sections and subsequent evaluation of mitochondrial changes under low power electron microscopy. This option will permit morphologic confirmation of suspected mitochondrial damage, should lactate clearance kinetics suggest such a possibility.

Quantitative evaluation of neuronal damage by light microscopy at 1000X magnification under oil was made. Coronally sectioned slides located at intervals corresponding to voxels analyzed for metabolic change will be evaluated for neuronal damage by published methods.(12,13). Neuronal analysis requires that a sampling strategy be employed which permits adequate characterization of the neuronal changes in each voxel without excessive sampling. To expedite the analysis the relatively rapid method of Eke et al 1990 (3) is being used.

The principal features used to classify neuronal damage include change in nuclear shape, smearing of the nuclear/cytoplasmic boundary, and total disintegration of the nucleus. We classify neurons Type I-IV where I is normal, II and III are intermediate forms and IV represents maximal injury, ie cell death (12, 13).

Type I and II neurons contain sharply delineated nuclei than can be readily differentiated from the surrounding cytoplasm. The nucleus is lighter than the surrounding cytoplasm and stands out with high contrast. Its round shape distinguishes normal Type I from the abnormally angular nucleus of Type II. Type III neurons have low nuclear-cytoplasmic contrast with an indistinct nuclear-cytoplasmic border. Type IV neurons have dark cytoplasm with no recognizable nucleus, though they sometimes contain nuclear remnants, often a surviving nucleolus. The Type IV neuron is now generally accepted as irreversibly damaged if fixation artifact has been excluded. We have validated this classification system, and analyzed conceptual and technical errors which may occur (12,13).

3. Results: One limitation to this method is the large amount of data acquired during such an experiment, typically several thousand brain spectra per animal (100 spectra per time point x 15-20 time points). To overcome this limitation we have developed a highly sophisticated data processing and analysis tool which allows us to automatically process all of the data. At this point in time all animal data has been collected however we are still completing the data processing and analysis. All brains have been sampled and sectioned and scanned for changes in optical density (which appear to correlate with regions of histologic damage). We are now in the process of carrying out the neuronal counting analysis, spectral processing and determination of the lactate content and clearance kinetics. All data analysis should be completed in approximately two to three months. However we have completed a limited analysis for five animals which demonstrates a strong correlation between histologic damage and slowed clearance of lactate during reflow. Preliminary findings suggest that the lactate level present at the conclusion of ischemia was not as strongly correlated with ischemic damage. Displayed in figure 1 are a series of brain sections, 1mm thick, spanning the brain in the anterior-posterior direction. The darker regions correlate to areas with a higher optical density which we have shown to be correlated with histologic damage. The locations of the acquired spectra are shown on the reconstructed brain map (top of figure) and the degree of damage from optical density measurements highlighted in dark green. The kinetics from each of the pixels is also displayed on the right and left sides of the figure. The damaged region (right) shows a clear bi-exponential clearance, with those regions showing damage having a second slower component to lactate clearance. Upon completion of the analysis of all animal data these results will be documented in a manuscript and forwarded to the Air Force for review.

IV REFERENCES

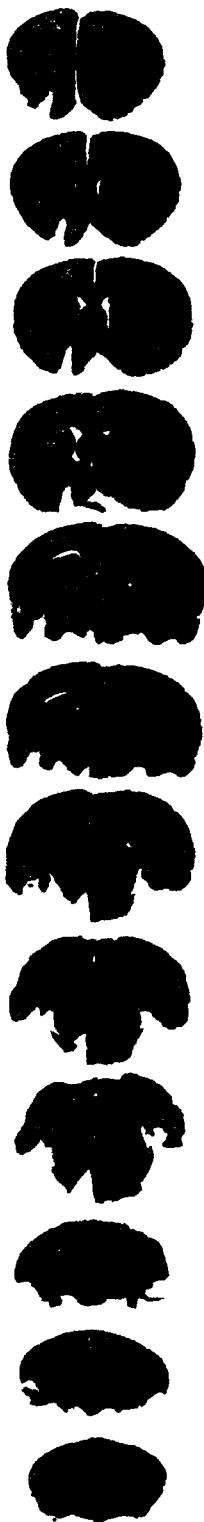
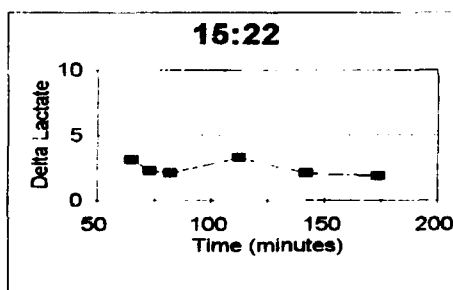
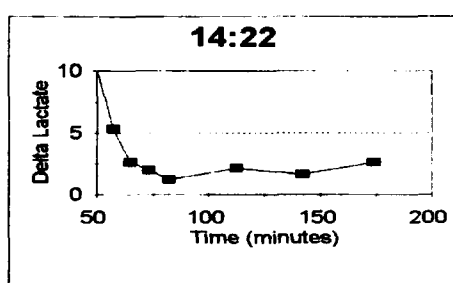
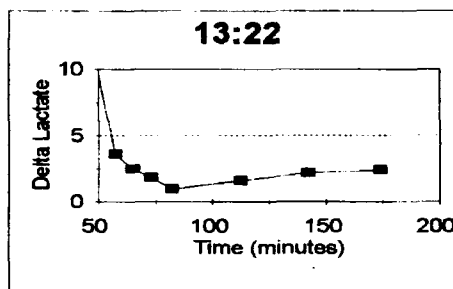
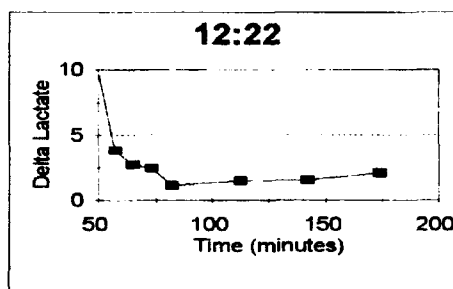
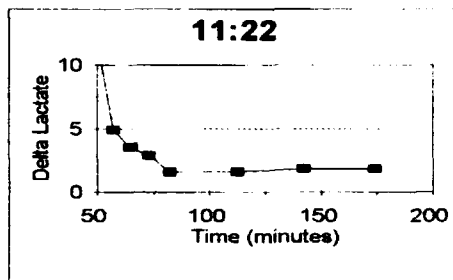
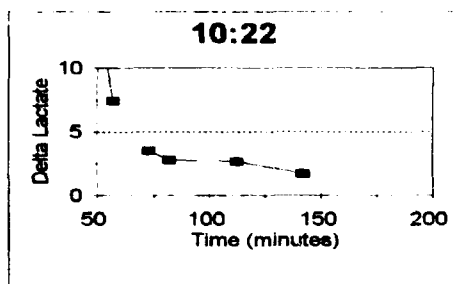
- 1) Conger KA, Hetherington HP, Tan MJ, Lou KL, Pohost GM and Halsey JH, Simulating Lactate Accumulation During Brief Repetitive Cerebral Ischemia, Proceedings of the Society of Magnetic Resonance in Medicine, #2158 Berlin Germany 1992.
- 2) Conger KA, Halsey JH, Tan MJ, Lou KL, Pohost GM and Hetherington HP, Concomitant EEG, Lactate and Phosphorous Changes During Repeated Brief Cerebral Ischemia, Proceedings of the Society of Magnetic Resonance in Medicine, New York, New York, 1993.
- 3) Hetherington HP, Halsey JH, Tan MJ, Lou KL, Pohost GM and Conger KA, Correlating EEG and Lactate Kinetics During Repeated Brief Cerebral Ischemia, Proceedings of the American Heart Association 1993.
- 4) HP Hetherington, MJ Tan, KL Lou, GM Pohost, JH Halsey and KA Conger, Evaluation of Lactate Production and Clearance Kinetics by ^1H NMR in a Model of Brief Repetitive Cerebral Ischemia, submitted J. Cerebral Blood Flow and Metabolism.
- 5) KA Conger, JH Halsey, MJ Tan, KL Lou, GM Pohost, and HP Hetherington, Concomitant EEG, Lactate and Phosphorous Changes During Repeated Brief Cerebral Ischemia, in preparation J. Cerebral Blood Flow and Metabolism .
- 6) Lowry OH, Passonneau JV, Hasselberger FX, Shulz DW, Effect of Ischemia on Known Substrates and Cofactors of the Glycolytic Pathway in Brain, J. Biol. Chem 239 18-30, 1964.
- 7) Drewes LR and Gilboe DD, Glycolysis and the Permeation of Glucose and Lactate in the Isolated , Perfused Dog Brain during Anoxia and Postanoxic Recovery, J. Biol. Chem., 248:2489-2496 1975.
- 8) Nilsson B, Norberg K, Nordstrom CH, Seisjo BK, Rate of Energy Utilization in the Cerebral Cortex of Rats. Acta Physiol. Scand. 93: 569-571 .
- 9) Eke A, Conger KA, Classifying cells from light microscopic bit features by binary logic,: Application to grade neuronal injury in cerebral ischemia. Lab. Invest. 61:243-252, 1989.
- 10) Eke A, Conger KA, Anderson ML, Garcia JH, Histologic assessment of neurons in rat models of cerebral ischemia. Stroke 21:299-304, 1990.
- 11) Cammermeyer J: Is the solitary dark neuron a manifestation of postmortem trauma to the brain inadequately fixed by perfusion? Histochemistry 1978, 56:97-115.

- 12) Eke A, Conger KA: Classifying cells from light microscopic bit features by binary logic: Application to grade neuronal injury in cerebral ischemia. Lab Invest 1989 61:243-252
- 13) Eke A, Conger KA, Anderson M, Garcia JH: Histologic assessment of neurons in rat models of cerebral ischemia. Stroke 1990, 21:299-304.

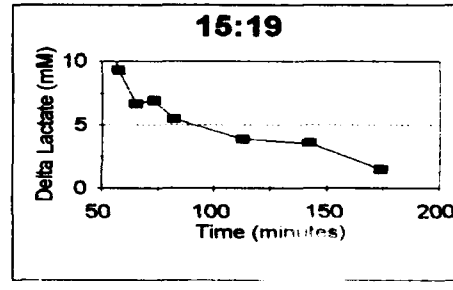
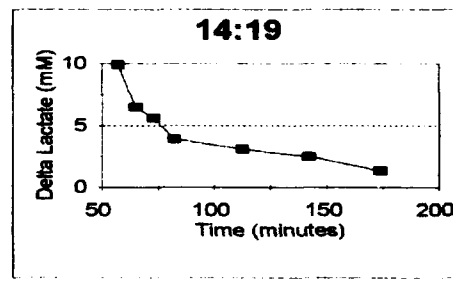
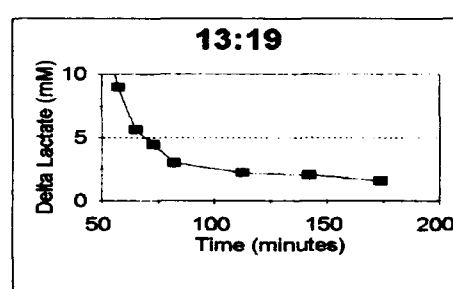
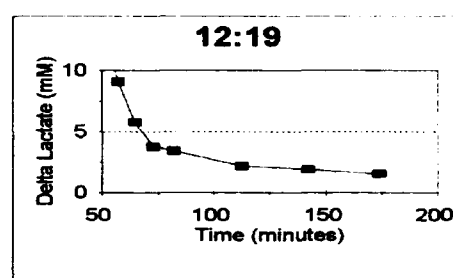
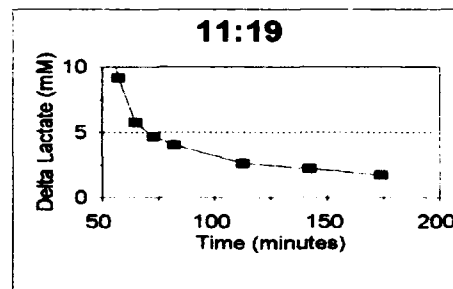
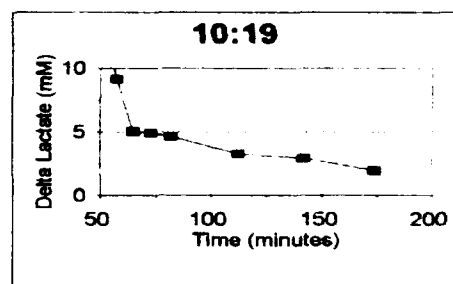
TABLE 1

Expt #	k_c	k_p	t_{sch}
1	0.119	5.71	20.2
2	0.113	3.72	22.2
3	0.103	4.88	21.0
4	0.181	4.44	22.7
5	0.134	5.27	20.7
6	0.179	4.59	19.8
7	0.158	3.82	21.0
Mean\pmstd	0.141\pm0.032	4.63\pm0.73	21.1\pm1.0

LEFT SIDE



RIGHT SIDE



Evaluation of Lactate Production and Clearance Kinetics by ^1H NMR in a Model of Brief Repetitive Cerebral Ischemia

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Summary: Pilots of high-performance aircraft are subject to repeated transient cerebral ischemia during high-gravitational stress maneuvers. Previously we have demonstrated that repeated episodes of transient cerebral ischemia and reflow are cumulative and lactate accumulations appear to be exponential. To evaluate the metabolic events determining the kinetics of lactate accumulation, and therefore the rates of substrate utilization, we have used *in vivo* ^1H nuclear magnetic resonance with a 5-s time resolution to measure lactate production and clearance. The individual rates for each animal were then used to predict the accumulation of lactate in the same animal during 30 episodes of ischemia and reflow. Lactate

accumulation was modeled as the balance between a zero-order production process during the ischemic period and a first-order clearance process. The predicted lactate accumulation showed excellent agreement with the observed time course, validating the predictive power of the simple model used. The highly reproducible nature of this model and its accuracy in predicting lactate accumulation should enable more accurate studies of the deleterious effects of lactate accumulation in cerebral ischemia by providing a highly reproducible means for generating a specific level of lactate accumulation. **Key Words:** *In vivo*—Ischemia—Lactate—Modeling—Nuclear magnetic resonance—Reperfusion.

Pilots of high-performance aircraft are subject to transient loss of consciousness due to gravitational stress-induced blackout. This loss of consciousness is due to cerebral ischemia, resulting from a failure of blood delivery to the brain due to the severe gravitational stress. To investigate the metabolic events associated with this form of ischemia, we have developed a rat model of brief repeated cerebral ischemia. Previously at a relatively low time resolution (1 min), we demonstrated that lactate production during 30 consecutive periods of reflow and ischemia follows an approximately exponential increase to a plateau level (Conger et al., 1993). To

evaluate the determinants of lactate production that give rise to this accumulation profile, we have made rapid serial measurements of lactate (5- or 10-s time resolution) by *in vivo* ^1H nuclear magnetic resonance (NMR) spectroscopy. We have used these measurements (a) to quantitate the ischemic production and clearance rates and (b) to model lactate accumulation during 30 sequential periods of ischemia and reflow using these measured rates.

METHODS

Animal model

Seven male Sprague-Dawley rats (200–240 g) were anesthetized with 70% N_2O , 29% O_2 , and 1% halothane, paralyzed with tubocurarine chloride, and mechanically ventilated. The external carotid and subclavian arteries were ligated. The right carotid artery was bidirectionally cannulated to monitor the carotid stump pressure (CSP; reflecting the cerebral perfusion pressure at the circle of Willis) and to measure the systemic pressure (Conger et al., 1993). Rectal and mouth temperatures were monitored throughout the experiment and maintained at 37°C by use of feedback-regulated (mouth temperature) heat-

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Abbreviations used: CSP, carotid stump pressure; NMR, nuclear magnetic resonance; T1 and T2, longitudinal and transverse relaxation time, respectively; TR, repetition time.

ing of a chamber surrounding the animal. Ischemia was induced by an inflatable occlusive cuff placed about the left common carotid artery. Inflation and deflation of the cuff were controlled by a hydraulic system linked to an IBM AT computer. Two ischemic protocols were used in each animal. A brief (60-s) sustained occlusion followed by 15 min of reflow was used to measure the rate of lactate production during ischemia. The repetitive ischemia protocol consisted of 30 consecutive 30-s (nominal-duration) ischemia and 30-s reflow periods, followed by 30 min of continuous reflow. The 1-min occlusion protocol was carried out before and after (in three animals) the repetitive ischemic protocol. Hematocrit, P_aO_2 , P_aCO_2 , pH, and blood glucose levels were determined immediately prior to the first ischemic protocol.

Biochemical methods

Following the postmortem determination of longitudinal (T1) and transverse (T2) relaxation time (see NMR Methods, below) the 2–3 mm of cerebral cortical tissue located directly under the coil was removed from the brain and frozen. Preparation of perchloric acid extracts and subsequent analysis of these extracts for lactate and creatine concentrations were by standard spectrophotometric methods (Lowry and Passonneau, 1972).

Electrode implants and measurement of cerebral blood flow (CBF)

In a separate series of experiments electrode arrays (four electrodes per array) were made from 90% platinum/10% iridium wire and had a 1-mm exposed tip. They were implanted in eight Sprague–Dawley rats anesthetized with halothane (2.5% for induction, 1.5% during surgery) in a small craniectomy 1 mm from the sagittal suture and 1 mm caudal to the bregma. The electrode array was placed with the electrode tips 1.5 mm deep into the cortex of the right hemisphere, and a silver/silver chloride reference electrode was implanted through a burr hole in the left hemisphere. Dental acrylic anchored the electrodes. Regional CBF was measured intermittently by hydrogen clearance at predetermined carotid stump pressures using reported methods (Boehme et al., 1988). Initiation and timing of hydrogen inhalation and washout, datum storage, and on-line flow analysis of CBF were performed by an IBM-AT computer using standard methods (Aukland et al., 1964; Haining et al., 1968; Boehme et al., 1988).

NMR methods

NMR data were collected using a 4.7-T, 40-cm bore Bruker Biospec system using a single tuned 8×12 -mm elliptical 1H surface coil placed directly on the skull. To minimize the acquired lipid signal, the scalp was removed and the temporal muscles were retracted (Behar et al., 1983). NMR spectra were acquired using a 136-ms spin-echo sequence and B_1 compensated semiselective pulses (Hetherington, 1988; Balschi et al., 1992). During the 60-s sustained ischemic periods, spectra were acquired with a 5-s time resolution using a repetition time (TR) of 1,250 ms and acquiring four averages per datum block. During the repetitive ischemia protocol and the first 10 min of continuous reflow, spectra were acquired with a 10-s time resolution (TR, 1,250 ms; eight averages). During the final 20 min of reflow, spectra were acquired every 30 s (TR, 1,250 ms; 24 scans). Data were processed using a convolution difference to remove broad water components and filtered using a Lorentzian-to-Gaussian transformation

prior to Fourier transformation. Resonance areas were determined by peak integration using NMR1 (New Methods Research Inc., Syracuse, NY, U.S.A.). Resonance areas were converted to millimolar values using a 11 mM value for cerebral creatine + phosphocreatine, determined in a parallel set of experiments, and correcting for the excitation profile of the pulses and differential T1 and T2 losses. T1 and T2 values for creatine and lactate (post-mortem) were determined using incremented spin-echo and inversion–recovery spin-echo sequences.

Modeling

Lactate accumulation during the repetitive ischemia period was modeled as the balance between lactate production at a constant rate ($d[\text{lactate}]/dt = k_p$) and a first-order clearance process ($d[\text{lactate}]/dt = -k_c[\text{lactate}]$). The value of k_p for each animal was determined from a 1-min continuous occlusion before the repetitive ischemia protocol. The value of k_c was determined from the reflow period following 30 cycles of the repetitive ischemia protocol. The exact duration of the ischemic period in each animal was determined from the carotid pressure recordings. Due to the small residual flow experienced during the ischemic period ($12 \text{ ml } 100 \text{ g}^{-1}\text{min}^{-1}$ at a carotid pressure of 10 mm Hg), lactate clearance (including wash-out and metabolism) was assumed to occur continuously throughout the ischemic and reflow intervals (see Results). The calculated values for k_p and k_c along with the ischemia duration for each animal were then used to predict the time course of lactate accumulation during the repetitive ischemia protocol.

RESULTS

Quantitation of 1H metabolite resonances

To convert the measured resonance areas to millimolar values, the T1 and T2 of creatine were measured in a separate set of four animals. Due to the uncertainties in spectral overlap from threonine, alanine, and lipids at the lactate resonance position, the lactate T1 and T2 were determined after sacrifice, when the lactate component dominates the resonance area. The T2 was measured using the same sequence and incrementing the total echo time from 68 to 374 ms at intervals of 34 ms (10 values). The T1 was determined using an initial inversion recovery sequence prior to the spin-echo sequence. The inversion delays used were, 10, 100, 300, 500, 700, 900, 1,200, 1,500, 2,000, and 5,000 ms. The correction factor for the excitation and refocusing profile was calculated using product operators (Balschi et al., 1992). The T1 and T2 values were measured to be $1,217 \pm 187$ and 189 ± 19 ms, for creatine + phosphocreatine and $1,235 \pm 24$ and 188 ± 24 ms for lactate. To evaluate the accuracy of these correction factors, perchloric acid extracts were made of the cortical regions of these four animals. The cortical creatine content was found to be 11.0 ± 0.4 mM. Using the correction factors determined from the relaxation measurements, and a 11.0 mM value

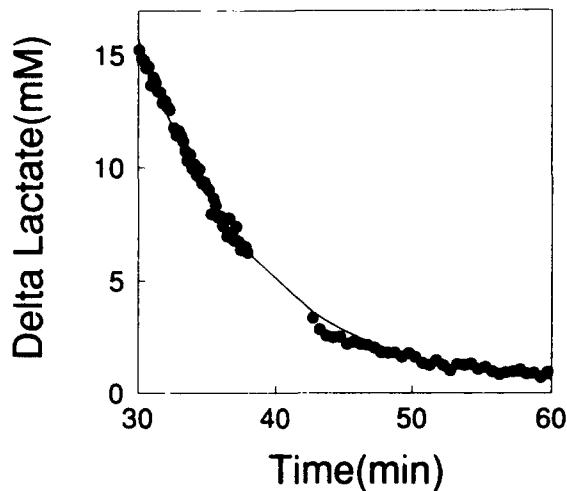


FIG. 1. Representative time course of lactate clearance. The observed values (filled circles) for lactate (delta lactate) are plotted as a function of the time after the beginning of ischemia. Thirty minutes corresponds to the beginning of the continuous reflow period. The lactate levels are reported as the difference in areas of the 1.33-ppm resonance from that observed in the control period. The first 8 min of the clearance data was acquired with a 10-s time resolution. The gap in the data from 38 to 41 min is the amount of time required for saving the NMR data. Measurements after this point in time are acquired with a 30-s time resolution. The fitted exponential clearance curve is indicated by the solid line. The clearance rate k_c was determined using a simplex algorithm and minimizing the squares of the differences of the observed and calculated data.

for creatine, the NMR-determined value for lactate was 23.5 ± 3.8 mM, in good agreement with the biochemically determined value of 24.1 ± 1.7 mM. The lactate resonance areas in the subsequent ischemia experiments were converted to millimolar values using these corrections and a creatine resonance area of 11.0 mM.

Lactate clearance

The effective rate of lactate clearance, including contributions from conversion to pyruvate and subsequent metabolism (Kuhr et al., 1988), transport of lactate across neurons (Assaf et al., 1990), and transport across the blood-brain barrier, was determined in each animal using the equation

$$[\text{lactate}](t) = [\text{lactate}]_0 \exp(-k_c t)$$

where $[\text{lactate}]_0$ is the lactate present at the beginning of the 30-min reflow period, t is the time in minutes, and k_c is in units of $1/\text{min}$. The lactate level is reported as a difference from the control period to minimize the effect of resonance overlap with other metabolites and lipids. Figure 1 is an example of the lactate clearance along with the fitted exponential. The measured clearance rates are reported in Table 1 along with the means (0.141 min^{-1}) and standard deviations ($\pm 0.032 \text{ min}^{-1}$) for all seven animals.

Lactate production

The lactate production rate was determined from a 1-min continuous occlusion in each animal prior to the repetitive ischemia protocol. Typical 5-s lactate spectra are displayed in Fig. 2a. To minimize the effect of lipid signals and contributions from alanine and threonine to the lactate resonance, the difference in the lactate resonance area from that measured in the control spectra prior to the 1-min occlusion was used to calculate the production rate. The lactate difference data were fit using a linear

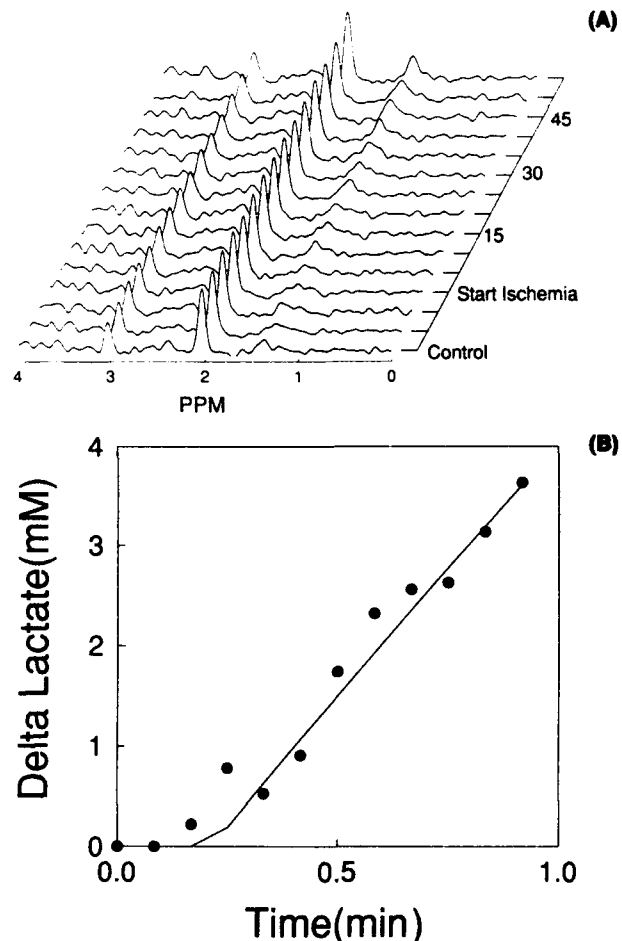


FIG. 2. A: Lactate spectra acquired with a 5-s time resolution, during the control period (first three spectra) and during 1 min of continuous ischemia. The starting time of the spectrum relative to the beginning of the ischemia is noted on the vertical axis in seconds. The observed resonances are as follows: lactate, 1.33 ppm; N-acetyl aspartate, 2.02 ppm; glutamate, glutamine, aspartate, and N-acetyl aspartate, 2.1–2.8 ppm; creatine + phosphocreatine, 3.03 ppm; and choline, 3.18 ppm. B: Lactate accumulation time course with the observed values (filled circles) for lactate (delta lactate) as a function of the time after ischemia in minutes. The modeled values for lactate accumulation including the clearance term are denoted by the line. The generation rate, k_p , was determined using a simplex algorithm and minimizing the squares of the differences between the observed and the calculated data.

production rate and a correction for lactate clearance using the differential equation

$$d[\text{lactate}]/dt = k_p - k_c[\text{lactate}]$$

where k_p is the production rate (mM/min). The value of k_c used was determined as described previously. Elimination of the correction term for lactate clearance resulted in a $4.7 \pm 1.7\%$ reduction in the production rates k_p . A typical datum set is displayed in Fig. 2b. Calculated values for k_p for all seven animals are listed in Table 1. The mean value reported here, including the clearance term (4.63 mM min^{-1}) is somewhat lower than that reported by Nilsson et al. (1975), $7.0 \text{ } \mu\text{mol g}^{-1} \text{ min}^{-1}$, possibly reflecting the anesthesia state or the small residual blood flow. In three animals rate measurements were made before and after the repetitive ischemic intervals, with the pre-to-post ratio of the calculated ratio of k_p being 0.99 ± 0.17 .

Determination of ischemic duration

Due to the finite time for inflation and deflation of the occlusive cuff, the actual ischemic periods were found to be shorter than 30 s and to vary slightly from animal to animal. Additionally, substantial lactate generation or anaerobic glycolysis will occur only after brain oxygen stores have been depleted. Lowry et al. (1964) have reported that lactate generation begins after a 3-s time delay in animals subjected to complete global ischemia. To account for the time delay in inflating the occluder and the subsequent time required to consume brain oxygen stores, the duration of the ischemic period was defined as the time during which the carotid pressure was within 1 mm Hg of the ischemic minimum value (Fig. 3) less 3 s. This correction shortened the mean ischemic duration (t_{isch}) to 21.1 ± 1.0 s and the individual times for each animal are reported in Table 1.

Duration of lactate clearance

To evaluate the effective duration of the lactate clearance period during the repetitive ischemia pro-

tol, CBF using the H_2 clearance method (Aukland et al., 1964; Haining et al., 1968; Boehme et al., 1988) was determined in eight animals subjected to the identical surgical preparation. The residual blood flow was found to be $12 \text{ ml } 100 \text{ g}^{-1} \text{ min}^{-1}$ at a carotid pressure of 10 mm Hg (Fig. 4). This residual flow is insufficient to support complete aerobic metabolism, however, it was assumed to be sufficient to support the clearance of lactate, through metabolism and washout. Therefore, lactate clearance was modeled as occurring throughout the reflow and ischemic periods.

Lactate accumulation during repetitive ischemia

The time course of lactate accumulation during the 30 consecutive ischemia/reflow periods was modeled using the rate constant, k_p and k_c , and the ischemic duration calculated from the carotid pressure measurements. The incremental change in lactate during each second of the ischemic (t_{isch}) reflow period and during the subsequent recovery period was calculated using the lactate generation rate (k_p) and clearance rates k_c

$$d[\text{lactate}] = k_p - k_c[\text{lactate}]$$

(during ischemia $t < t_{\text{isch}}$)

$$d[\text{lactate}] = -k_c[\text{lactate}]$$

(during reflow $t > t_{\text{isch}}$)

$$d[\text{lactate}] = -k_c[\text{lactate}]$$

(during continuous reflow)

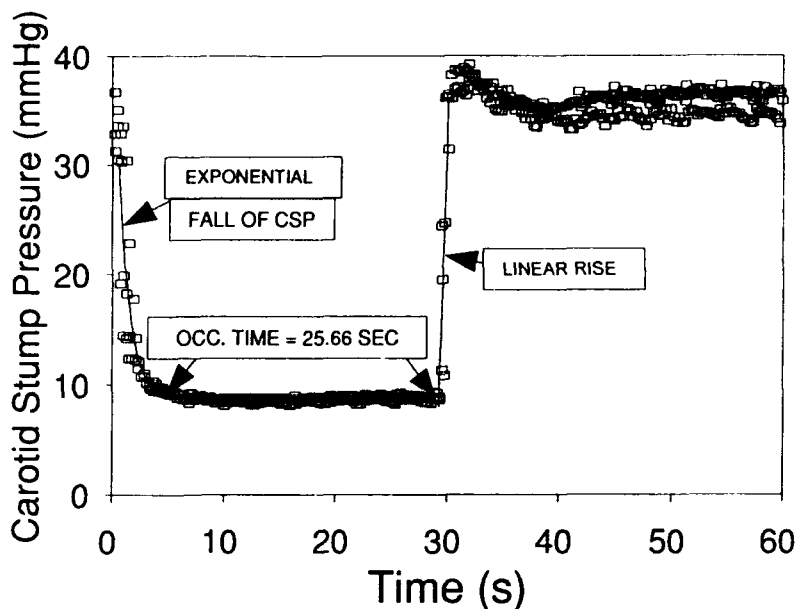
To evaluate the accuracy of the calculated time courses, the observed data at the conclusion of the reflow period, calculated data, and calculated data assuming $\pm 15\%$ errors in the production rate constants (Table 1) are displayed for all seven animals in Fig. 5. All animals showed excellent agreement, with nearly all measured values during the repetitive ischemic period lying within the 15% error bounds. Additionally, six of the seven animals showed excellent agreement during the recovery period, indicating that the lactate level during the final ischemic episode calculated was in good agreement with the observed value. Only animal 2 showed a poor correlation during the recovery period, due to a nonexponential clearance during the latter portion of the recovery period. Assuming error bounds of 15% in the clearance rate provides similar limits, and they are not shown for the sake of clarity.

The high sensitivity of the shape and absolute level of lactate accumulation to the clearance rate, production rate, and ischemic duration and the agreement of the model and observed data suggest

TABLE 1. Kinetic parameters

Experiment no.	k_c (min^{-1})	k_p (mM/min)	t_{isch} (s)
1	0.119	5.71	20.2
2	0.113	3.72	22.2
3	0.103	4.88	21.0
4	0.181	4.44	22.7
5	0.134	5.27	20.7
6	0.179	4.59	19.8
7	0.158	3.82	21.0
Mean \pm SD	0.141 ± 0.032	4.63 ± 0.73	21.1 ± 1.0

FIG. 3. The CSPs from all 30 occlusions from a typical animal were overlaid as a function of their time during the individual ischemic episode. The fall in CSP was modeled as an exponential, whereas the rise in CSP accompanying the release of the occluder was modeled as a linear process. The duration of the occlusion was calculated as the difference between the two fits at a point 1 mm Hg above the minimum CSP. The minimum CSP was determined from the exponential fit.



that both the model used and the rates measured are highly accurate (within 15%). Elimination of the clearance term during the ischemic interval and iteration of the model every second resulted in a substantially higher predicted lactate level, $141 \pm 14\%$ greater than the final lactate level during the last ischemic episode. When clearance is assumed to occur during the ischemic intervals, the calculated lactate levels and those observed during the final ischemic period were in exact agreement, $100 \pm 10\%$.

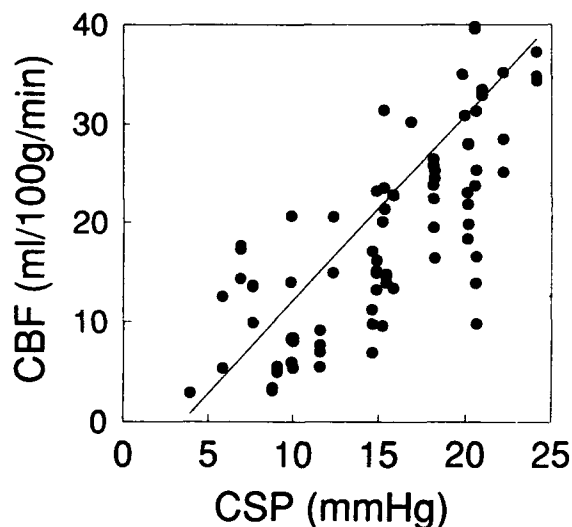


FIG. 4. The relationship between CBF and CSP for eight rats. The data were obtained from eight rats implanted with cortical H_2 electrodes. The CBF was determined by H_2 clearance within 5 to 10 min after reduction of the CSP to the specified level. The CSP was maintained at that level by servocontrolled compression of the left carotid artery with a balloon occluder.

Correlation with blood glucose

Initial blood glucose levels were correlated with the final levels of lactate reached at the conclusion of the ischemic period and are listed in Table 2. The calculated correlation coefficient was -0.26 , indicating a poor correlation between initial glucose levels and final lactate levels.

DISCUSSION

In this report we have demonstrated that the accumulation of lactate during a brief repetitive ischemia protocol can be accurately modeled ($<15\%$ error) using rate constants determined on an individual animal basis. Thus the simple model of lactate production at a constant rate during each ischemic period, and clearance throughout the reflow and ischemic period, provides an excellent model for predicting the amount of lactate accumulated during the protocol. The observed lactate production during each ischemic period appears to occur at the same rate as during a continuous 1-min ischemic episode and these rates are lower than the maximum glycolytic rates observed by Lowry et al. (1964) in a model of complete global ischemia. This, combined with the poor correlation between the initial glucose levels and the final levels of lactate reached, suggests that lactate production in this repetitive ischemia model is not limited by glucose or other substrate availability. This suggests that glucose replenishment during the reflow period is sufficient to support the elevated level of anaerobic activity during the next ischemic interval. This is in contrast to total continuous global ischemia models,

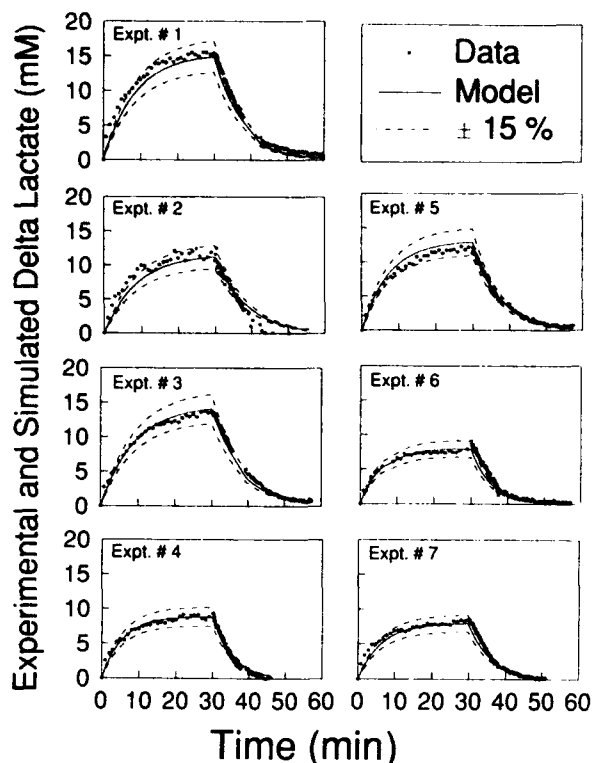


FIG. 5. The observed (filled circles) and calculated lactate data (solid and dashed lines) from the seven experimental animals. The lactate level (delta lactate; mM) is plotted as a function of the time in minutes after the beginning of the repetitive ischemia protocol. The continuous reflow period was initiated 30 min after beginning the repetitive ischemic protocol. The solid line indicates the calculated lactate time course using the experimentally determined values of k_p and k_c . The calculated time courses assuming a $\pm 15\%$ error in the production rate of k_p are indicated by the dashed lines.

where the amount of lactate produced is dependent on the brain glucose and glycogen stores immediately prior to the induction of ischemia.

Although the model proposed is quite simple, obviously ignores lactate production during the reflow period, and lumps the variety of mechanisms that can contribute to lactate clearance, metabolism via conversion to pyruvate for subsequent oxidation,

TABLE 2. Initial blood glucose and ischemic lactate levels

Experiment no.	Glucose (mM)	Lactate (mM)
1	8.6	15.4
2	10.9	10.8
3	8.1	13.7
4	6.4	9.3
5	12.3	11.4
6	7.9	9.1
7	14.5	8.5

and transport across the blood-brain barrier and neurons into a single exponential rate constant, the excellent agreement between the calculated and the observed lactate accumulation data validates the predictive power of the model. Therefore this protocol has the potential of providing a highly reproducible state of lactate elevation, which should allow more detailed studies of the mechanisms by which lactate elevations (acidosis, osmotic alterations) contribute to ischemic damage. Additionally, since the lactate elevation occurs over a fairly long time period, tens of minutes, time-course correlations with measurements of ATP, phosphocreatine, and inorganic phosphate (less NMR-sensitive compounds requiring longer acquisition times for an adequate signal-to-noise ratio) are also possible.

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REFERENCES

- Assaf HM, Ricci AJ, Whittingham TS, LaManna JC, Ratcheson RA, Lust WD (1990) Lactate compartmentation in hippocampal slices: evidence for a transporter. *Metab Brain Dis* 5:143-154
- Aukland K, Bower BF, Berliner RW (1964) Measurement of local blood flow with hydrogen gas. *Circ Res* 14:164-187
- Balschi JA, Hetherington HP, Pohost GM (1992) Water suppressed one dimensional ^1H NMR spectroscopic imaging of myocardial metabolites in vivo. *Magn Reson Med* 25:180-186
- Behar KL, den Hollander JA, Stromski ME, Ogino T, Shulman RG, Petroff OAC, Prichard JW (1983) High resolution ^1H nuclear magnetic resonance study of cerebral hypoxia in vivo. *Proc Natl Acad Sci USA* 80:4945-4948
- Boehme RJ, Conger KA, Anderson ML (1988) Computer regulated constant pressure ischemia in the rat: the animal model. *J Cereb Blood Flow Metab* 8:236-243
- Conger KA, Halsey JH, Tan MJ, Luo KL, Pohost GM, Hetherington HP (1993) Concomitant EEG, lactate and phosphorous changes by ^1H and ^{31}P NMR spectroscopy during repeated brief cerebral ischemia (submitted for publication)
- Haining JH, Turner D, Pantall RM (1968) Measurement of local cerebral blood flow in the unanesthetized rat using a hydrogen clearance method. *Circ Res* 23:313-324
- Hetherington HP (1988) *The Design and Application of Multi-pulse NMR Techniques to in Vivo ^1H NMR*, Ph.D. thesis, New Haven, CT, Yale University
- Kuhr WG, van den Berg C, Korf J (1988) In vivo identification and quantitative evaluation of carrier mediated transport of lactate at the cellular level in the striatum of conscious, freely moving rats. *J Cereb Blood Flow Metab* 8:848-856
- Lowry OH, Passonneau JV (1972) *A Flexible System of Enzymatic Analysis*, New York, Academic Press, pp 174-177
- Lowry OH, Passonneau JV, Hasselberger FX, Schulz DW (1964) Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J Biol Chem* 239:18-30
- Nilsson B, Norberg K, Nordstrom CH, Siesjo BK (1975) Rate of energy utilization in the cerebral cortex of rats. *Acta Physiol Scand* 93:569-571

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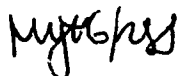
Dear Dr. Conger:

Re: MS 93-2460R2: "Concomitant EEG, lactate and phosphorous changes by ^1H and ^{31}P NMR spectroscopy during repeated brief cerebral ischemia"

I am pleased to tell you that your revised manuscript is now acceptable for publication and will be forwarded to the publisher in due course.

With best wishes.

Sincerely,



Mordecai Y.-T. Globus, M.D.
Assistant Editor

MYTG:rss

Manuscript No. 93-2460 Revised 4/25/94

**Concomitant EEG, Lactate and Phosphorous Changes
by ^1H and ^{31}P NMR Spectroscopy
During Repeated Brief Cerebral Ischemia.**

Karl A. Conger¹, James H. Halsey², Kang-Li Luo¹, Min-Jie Tan¹, Gerald M. Pohost³, and
Hoby P. Hetherington³.

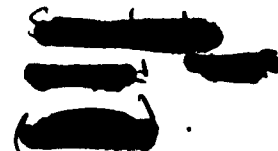
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Accepted J. Cerebral Blood Flow
and Metabolism



SUMMARY

Pilots of high performance aircraft are subject to transient loss of consciousness due to cerebral ischemia resulting from sudden high gravitational stress. To assess the effects of high gravitational stress induced blackout on cerebral metabolism and electrical function we have developed an animal model in which global cerebral ischemia is produced repeatedly at short intervals. Rats were prepared by ligation of subclavian and external carotid arteries and the right carotid artery was cannulated bidirectionally to measure Circle of Willis and systemic pressures. Ischemia was induced by inflation of an occluder about the left carotid artery. Interleaved ^{31}P and ^1H NMR spectra were acquired on a 4.7T Biospec system simultaneously with EEG recordings. We report results from 20 experiments of 30 minutes duration in which rats were subject to 30 one minute ischemia:reflow cycles of 10I:50R, 20I:40R, 30I:30R, and 40I:20R (numbers are seconds of ischemia (I) and reflow (R) respectively during each one minute cycle). During ischemia the graded delivery of the ischemic insult permitted direct correlations between 2-5 Hz and 7-20 Hz EEG activity and progressive changes in pH, lactate, ATP, PCr and Pi. The best correlations were found between EEG activity and pH and PCr, correlation coefficients ranged between 0.93 to 0.95. Loss in EEG activity was observed without significant sustained energy loss in all but the most severe cycle.

Key Words: Ischemia, Reperfusion, Lactate, EEG, Phosphocreatine, ATP, Brain

Running Title: EEG, Lactate and Phosphorous Changes"

INTRODUCTION

During high gravitational stress maneuvers, pilots of high performance aircraft can lose consciousness due to cerebral ischemia. Although typically brief in nature, these episodes may be repetitive. The goal of this work was to evaluate the temporal progression of the metabolic and functional changes accompanying repetitive brief cerebral ischemic episodes. Repeated brief cerebral ischemia presents the special opportunity to study **graded** delivery of ischemic insult over a specified time period, in contrast to the classical situation of acute stroke in which an all-or none insult is delivered immediately. Magnetic Resonance Spectroscopy (MRS) is an ideal method for monitoring this evolution by in vivo serial measurements.

To evaluate the time course of the metabolic changes serial interleaved ^1H and ^{31}P NMR spectra were collected while rats were subjected to 30 one minute episodes of cerebral ischemia and reflow followed by thirty minutes of continuous reflow. The severity of the ischemia was modulated by altering the durations of the ischemic and non-ischemic durations during the one minute ischemic:reperfusion interval. To evaluate the effect of the repeated ischemic episodes on brain function, slow (2-5 Hz) and fast (7-20Hz) components of the EEG were recorded throughout the ischemic and reflow periods.

METHODS

NMR Animal Model

Male Sprague Dawley rats (200-240g) were anesthetized with 70% N₂O, 29% O₂, and 1% Halothane, paralyzed with tubocurarine chloride and mechanically ventilated. The external carotid and subclavian arteries were ligated. The right carotid artery was bi-directionally cannulated for monitoring the carotid stump pressure (CSP, reflecting the cerebral perfusion pressure at the Circle of Willis) and for measurement of mean arterial blood pressure (MABP). Rectal temperatures were monitored throughout the experiment and maintained at 37°C by use of feedback regulated heating chamber surrounding the animal. Ischemia was induced by inflation of an occlusive cuff placed about the left carotid artery (Type OC-2A - In Vivo Metric, Healdsburg, CA). Inflation and deflation of the cuff was controlled by a hydraulic system linked to a IBM AT computer (figure 1). EEG activity was monitored between two blunt platinum-iridium wire electrodes which were placed bilaterally through burr holes in the bone. These electrodes were fixed in their extradural position adjacent to the lateral cortex with cyanoacrylic and dental acrylic. Within the RF shielded room, the EEG voltage was amplified, converted to a frequency proportional to voltage and optically coupled to a frequency to voltage converter. This additional isolation effectively broke the antenna effect which had previously seriously compromised the quality of the ³¹P spectra. The EEG signal was subsequently actively filtered to obtain slow (2-5Hz) and fast (7-20Hz) components (Halsey et al., 1991). Quantitative EEG components, MABP, CSP, and rectal temperature were digitized every second throughout the experiment. Hematocrit, P_aO₂, P_aCO₂ and pH were determined immediately prior to ischemia.

Each experiment consisted of: 1) an initial period of 10 minutes for control measurements; 2) followed by 30 cycles of brief cerebral ischemia and reflow (each cycle lasting one minute); 3) concluding with 30 minutes of continuous reflow. The severity of the ischemia was modulated by altering the durations of the ischemic and non-ischemic durations during the one minute ischemic:reperfusion interval. Four protocols were utilized in 20 studies:

10I:50R, 10 seconds of ischemia followed by 50 seconds of reflow (n=6), 20I:40R (n=6), 30I:30R (n=4) and 40I:20R (n=4).

NMR Methods

NMR data was collected using a 4.7T 40cm bore Bruker Biospec system using a two coil $^1\text{H}/^{31}\text{P}$ detector. An elliptical ^{31}P surface coil (8x12mm) was placed directly upon the exposed skull. The ^1H coil, was placed immediately above the ^{31}P coil and consisted of two 10mm circular loops connected in a butterfly configuration. The butterfly configuration minimizes coupling between the coils. To minimize the acquired lipid signal, the scalp was removed and the temporal muscles retracted (Behar et al., 1983). NMR spectra were acquired using a 136 msec. spin echo sequence with B_1 compensated semi-selective pulses (Hetherington, 1988; Balschi et al., 1992). ^{31}P spectra were acquired using a pulse acquire sequence with a non-selective square pulse. ^1H and ^{31}P spectra were acquired simultaneously by interleaving ^1H and ^{31}P acquisitions on a scan by scan basis. The data was acquired in one minute bins, (60 scans at a 1 second repetition time for both nuclei). ^{31}P spectra were summed in a moving average of four adjacent one minute measurements to improve the S/N. ^1H data was processed using a convolution difference to remove broad water components and filtered using a lorentz to gaussian transformation prior to fourier transformation for resolution enhancement. ^{31}P spectra were processed using a convolution difference to eliminate the broad bone signal and 20Hz of exponential broadening to improve the S/N. Resonance areas were determined by peak integration using NMR1 (New Methods Research Inc. Syracuse, New York). ^1H resonance areas were converted to millimolar values using a 11mM value for cerebral (phosphocreatine plus creatine) as an internal standard, (determined in a parallel set of experiments), and corrected for the spectral efficiency of the semi-selective pulses and T1 and T2 losses (Hetherington et al. 1993). The cerebral pH was determined using the equation (Petroff et al., 1985a):

$$\text{pH} = 6.77 + \log [(P_i \text{ shift } -3.29) / (5.68 - P_i \text{ shift})]$$

RESULTS and DISCUSSION

Carotid Pressure and EEG activity

The computer controlled occluder system provided a highly reproducible ischemic insult with minimal occlusion to occlusion variation in the carotid stump pressure during each ischemic episode. Typical systemic and carotid stump pressures during the 4 ischemic protocols are displayed in figure 2. One to two minutes prior to the first ischemic-reperfusion cycle halothane anesthesia is increased to approximately 1.5%. This increased anesthetic level, prevents or limits CSP increases during occlusions. In the illustrated experiment the first two occlusive pressures (CSP) are higher than subsequent pressures obtained during the remainder of the experiment in which the animal is fully equilibrated to the higher anesthetic level. The carotid stump pressure during the ischemic period was typically 7-10mmHg with variations of approximately 1-2mmHg seen for a given animal, with CSP reperfusion pressures of 28 to 35 mmHg. CSP values of 25 mmHg correspond to the bottom of the autoregulatory plateau for normotensive animals (Fujishima and Omae, 1976). Unreported experiments from our laboratory studying this model with implanted platinum electrodes polarized at -500 mv, to detect tissue oxygen levels, have shown that intermittent reperfusion at a CSP pressure of 25 to 30 mmHg is a sufficient pressure to permit brain tissue to return to normoxia or hyperoxia during each reperfusion cycle. During reperfusion halothane levels are restored to 1% levels and both MABP and CSP recover rapidly to pre-ischemic levels.

Both the slow (2-5 Hz) and fast (7-20 Hz) activity declined during each ischemia:reflow cycle in the 20I:40R, 30I:30R and 40I:20R animals (figure 3). Minimal changes were seen in the 10I:50R animals, most likely due to the shortness of the interval and the finite time required for cuff inflation and consumption of brain oxygen stores. To facilitate the analysis of long term trends, the EEG data was averaged over the one minute ischemia/reflow episode and for all animals in each group (figure 4). In the 10I:50R animals the average fast

activity was unchanged and an increase in slow activity was seen suggesting that partial cortical cholinergic deafferentation may have occurred (Gloor et al., 1977; Steriade et al., 1990). Fast and slow activity declined in the 20I:40R, 30I:30R, and 40I:20R protocols, with fast activity decreasing more than 80% in the 40I:20R protocol. The 20I:40R animals following an initial rapid reduction in fast activity reached an apparent steady state at approximately 60% of control activity. Slow activity in these animals was also found to decline but after 10 minutes slow recovery was seen. In the 30I:30R and 40I:20R animals, the decrease in average EEG activity was found to be progressive in successive ischemic episodes. This trend continued until the EEG collapsed in the 40I:20R animals. Upon reflow all groups showed a recovery of fast and slow activity reaching levels near or above control values by the end of a thirty minute reflow period.

Therefore, the 10I:50R protocol appears to establish a threshold for ischemic duration in this model below which significant changes in fast EEG activity do not occur. This minimum interval most likely reflects the amount of time required to establish the ischemia (several seconds for balloon inflation) and deplete brain oxygen stores (Lowry et al., 1964). The 20I:40R animals establish an apparent steady state of decreased EEG, such that the effects of each ischemic episode on EEG are balanced by each reflow period. In contrast, the 30I:30R and 40I:20R animals show an incremental decline throughout the thirty episodes of ischemia in EEG activity reflecting the inability of the brain to compensate for the cumulative effects of each new ischemic period. Specifically, the amount of fast EEG activity during each reflow period was found to decline. Thus the 30I:30R animals set a threshold for a slow progressive decline in EEG activity.

ATP, PCr and P_i

Displayed in figure 5 are representative ^{31}P spectra from a 40I:20R animal: during control, at the conclusion of the thirty episodes of ischemia and after thirty minutes of reflow. Similar to the EEG, the 10:50 ischemic protocol did not produce a significant decrease in PCr (figure 6). However, significant declines in PCr content relative to the control period values

were observed in the other groups. As expected the longer ischemic intervals resulted in greater decline in PCr, with the 40I:20R protocol producing a 75% decline in PCr. The shape of the PCr content curves is qualitatively similar to that of the EEG activity. The 20I:40R animals showed a rapid decline to a plateau level (75% of control) whereas the 30I:30R and 40I:20R animals showed initial rapid decreases followed by continued slow progressive declines. All animals showed near complete PCr recovery at the end of the thirty minutes of reflow.

Significant changes in average ATP were visualized only in the 40I:20R animals. This is consistent with a model where ATP is preserved at the expense of PCr (Swaab and Boer, 1972). Therefore the 50% average decrease in PCr seen in the 30I:30R animals does not appear to be sufficient to induce a significant change in the average ATP content, despite substantial changes in the EEG. This effect may reflect the averaging nature of the acquisition. Specifically, short term reductions in ATP during each ischemic interval may occur which might not appear as significant decreases in the 4 minute ischemia:reflow average data.

Cerebral P_i levels are reported as a fraction of the preischemia PCr content. Similar to that seen in the ATP data a significant increase in P_i content is visualized only in the 40I:20R animals, where substantial ATP hydrolysis augments the PCr dephosphorylation. However, the change in the 40I:20R group is substantial, displaying an increase of greater than 3 fold over control.

Lactate and pH

Displayed in figure 7 are one minute spectra acquired during the control period, at the conclusion of ischemia and at the conclusion of reflow in a 40I:20R animal. The resonance at 1.33 ppm has contributions from extracerebral lipids, lactate, threonine, alanine and lactate. To minimize the effects of these contaminants, the lactate data is reported as a difference in the 1.33 ppm resonance area from the average control value. Since contributions from extracerebral lipids should not be changing during the experiment and changes in lactate in millimolar quantities should dominate the changes in alanine and threonine, the difference data should provide a good estimate of the lactate accumulation induced by the ischemia.

Displayed in figure 8 are average time courses of the lactate changes for the four groups of animals. In this study, lactate proved to be the most sensitive indicator of ischemia, showing significant elevations in all four groups. As a symmetrical reflection to the decreases seen in EEG, lactate levels in the 20I:40R group showed a rapid increase to a plateau level. The plateau level resulting from a balance between exponential lactate clearance and linear lactate generation rates (Hetherington et al., 1993). Lactate changes in 30I:30R and 40I:20R groups showed rapid initial elevations followed by slow progressive increases. Despite the large changes in lactate observed in 40I:20R animals, approximately 18mM, lactate clearance during the subsequent reflow period was complete, with all animals returning to near control levels.

The time course of pH changes were qualitatively similar to those seen with lactate. The observed pH changes ranged from 7.15 in the control period to 7.1, 7.0, 6.8 and 6.6 at the conclusion of the ischemic episodes in the 10I:50R, 20I:40R, 30I:30R and 40I:20R animal groups, respectively. Again all animals returned to control values during the recovery period. Correlation of the pH and lactate data revealed a general trend towards decreasing pH with increasing lactate ($r=0.93$). However, the data were found to segregate according to duration of reflow cycle with decreased pH being associated with shorter reperfusion times at identical lactate levels.

EEG - Metabolic Correlations

Significant changes in Pi, pH and PCr content were found in all animals that approximately paralleled the decreases in EEG activity. To evaluate the relationship with these metabolic (Pi, pH and PCr) and functional variables (fast and slow components of the EEG), EEG was plotted as a function of Pi, pH and PCr (figure 9). There was a good correlation between the exponential increase in Pi concentration and the decline in both measures of EEG activity. Excellent linear correlations (0.93 to 0.95) were seen between the pH and PCr levels in these animals.

CONCLUSIONS

EEG was shown to decline in this model and be most strongly correlated with changes in pH and PCr (figure 9). The excellent correlation between pH and EEG suggests that EEG activity may be more affected by pH (figure 9) than by lactate levels. The curious differences between groups in pH at identical lactate levels deserves additional study (figure 8). Since the 20 second reperfusion period may be inadequate for clearing CO₂, and the pH differences are of the right magnitude to be associated with brain tissue CO₂ differences (Petroff et al., 1985b), this difference may reflect different reperfusion times between groups (figure 8). However, as the 40I:20R group also had significant ATP reduction not present in the other groups, it is possible that the generation of protons by ATP hydrolysis produces the noted pH differences (Erecinska and Silver, 1989) or that both effects contribute to the observed differences (figure 8).

Thus, we have demonstrated that brief repetitive ischemias can produce progressive highly reproducible declines in PCr, pH and increases in Pi and lactate. These changes are cumulative and can cause significant "average" changes in EEG which persist into the individual reflow periods. The severity of these changes is easily modulated by altering the ratio of the ischemic to reflow period during each episode. Unlike global ischemia models, due to the "gated" nature of the ischemia, the relatively slow time course of the metabolic and electrical changes permits the measurement of these processes by in vivo NMR. Additionally, the highly reproducible nature of the response and the ability to modulate the ischemia offers significant advantages for investigating metabolic thresholds for ischemic damage. The ischemia appears to be fully reversible, all metabolic and functional correlates returned to control levels, however histologic evaluations were not performed in these animals. Studies investigating differences in regional metabolic sensitivity and histologic correlations are currently underway.

ACKNOWLEDGMENT

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REFERENCES

- Balschi JA, Hetherington HP and Pohost GM (1992) Water suppressed one dimensional ^1H NMR spectroscopic imaging of myocardial metabolites in vivo. *Mag. Reson. in Medicine*, 25:180-186.
- Behar KL, den Hollander JA, Stromski ME, Ogino T, Shulman RG, Petroff OAC, Prichard JW (1983) High resolution ^1H nuclear magnetic resonance study of cerebral hypoxia in vivo. *Proc Natl Acad Sci USA* 80:4945-4948.
- Erecinska M and Silver IA (1989) Review: ATP and Brain Function. *J Cereb Blood Flow Metab* 9:2-19.
- Fujishima M, Omae T (1976) Carotid back pressure following bilateral carotid occlusion in normotensive and spontaneously hypertensive rats. *Experientia* 32:1021-1022.
- Gloor P, Ball G, Schaul N (1977) Brain lesions that produce delta waves in the EEG. *Neurology* 27: 326-333.
- Halsey JH, Conger KA, Garcia JH, Sarvary E (1991) The contribution of reoxygenation to ischemic brain damage. *J Cereb Blood Flow Metab* 11:994-1000.
- Hetherington HP, Tan MJ, Luo KL, Pohost GM, Halsey JH, and Conger KA (1994) Evaluation of Lactate Production and Clearance Kinetics by ^1H NMR in a Model of Brief Repetitive Cerebral Ischemia. *J Cereb Blood Flow Metab* (Accepted Dec. 11, 1993).

- Hetherington HP (1988) "The design and application of multipulse NMR techniques to in vivo ^1H NMR" PhD Thesis, Yale University.
- Lowry OH, Passonneau JV, Hasselberger FX, Schulz DW (1964) Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J Biol Chem* 239, 18-30.
- Petroff OAC, Prichard JW, Behar KL, Rothman DL, Alger JR, den Hollander JA, and Shulman RG (1985a) Cerebral intracellular pH by ^{31}P nuclear magnetic resonance spectroscopy. *Neurology* 35:781-788.
- Petroff OAC, Prichard JW, Behar KL, Rothman DL, Alger JR, and Shulman RG (1985b) Cerebral metabolism in hyper- and hypocarbia: ^{31}P and ^1H nuclear magnetic resonance studies. *Neurology* 35:1681-1688.
- Steriade M, Gloor P, Llinas RR, Lopes da Silva FH (1990) Basic mechanisms of cerebral rhythmic activities. *Electroenceph. Clin. Neurophysiol.* 76: 481-508.
- Swaab DF, Boer K (1972) The presence of biologically labile compounds during ischemia and their relationship to the EEG in rat cerebral cortex and hypothalamus. *J Neurochem* 19:2843-2853.

LEGENDS

Figure 1. Block diagram of the components of the occlusion system. The computer monitors the carotid stump pressure (CSP) and systemic blood pressure (MABP) via the right carotid artery. Temperature is monitored from a rectal probe. The ischemia is induced by inflating the cuff placed about the left carotid artery. Pressure within the cuff is maintained by the pump and regulated through the output of the transducer. The cuff is deflated by opening the valve.

Figure 2. The 60 minute time course of MABP and CSP changes during a 40:20 experiment is shown. Note the marked rise in MABP (cerebral ischemia response) associated with each carotid occlusion and the diminution of this response with time. The dependency of carotid stump pressure on systemic pressure during both occlusion and reperfusion is

evident. The inset 0 to 4 minute windows provide better visualization of the initial time course in this experiment and three other experiments with different ischemic:reflow times.

Figure 3. The 60 minute time course of slow and fast EEG change during a typical 40I:20R experiment is shown. Note the precipitous collapse and recovery of activity during each ischemic:reflow cycle and the cumulative loss of activity. The inset 0 to 4 minute windows provide better visualization of the initial time course of change in this experiment and views of three other experiments with different ischemic:reflow times. The time course of change in the slow and fast EEG components is quite similar in all these studies.

Figure 4. The illustration depicts the time course of change in both slow and fast EEG activity using one minute means \pm SD. In the 10I:50R animals a net increase in slow activity was seen during occlusion. In general, fast and slow activity declined in the 20I:40R, 30I:30R, and 40I:20R protocols reaching fairly steady state levels of reduction by 15 to 20 minutes. However, the 20I:40R animals showed a recovery in slow activity after 10 to 15 ischemic:reperfusion cycles. Upon reflow all groups recovered fast and slow activity by the end of the reflow period, with slow activity recovering most rapidly.

Figure 5. Displayed are representative ^{31}P spectra from a 40I:20R animal: during control, at the conclusion of the thirty episodes of ischemia:reflow and after thirty minutes of recovery. Particularly noteworthy is the marked loss of PCr (-2.35 ppm) and increase in inorganic phosphorous (2-3 ppm) following 30 cycles of ischemia:reflow which return to control levels following 30 minutes of recovery.

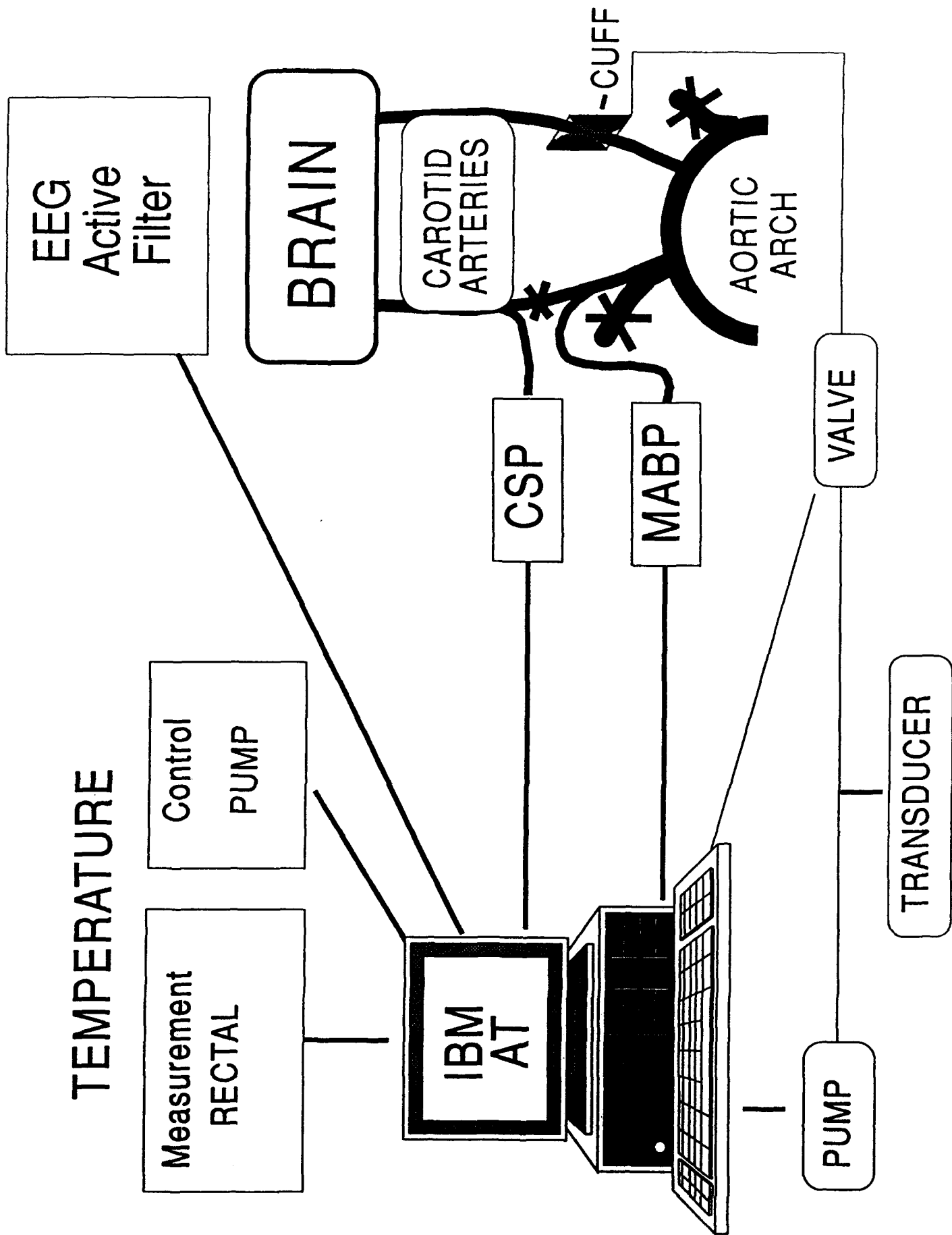
Figure 6. The illustration depicts the time course of change in PCr, ATP and P_i concentrations using four minute running averages \pm SD. The 10I:50R animals showed no changes in PCr, ATP and P_i concentrations during occlusion. PCr declined in the 20I:40R, 30I:30R, and 40I:20R protocols reaching fairly steady state levels of reduction by 10 minutes of

ischemia:reflow. Only the 40I:20R protocol produced significant ATP reduction and P_i elevation. All metabolite values returned to control levels during the subsequent recovery period.

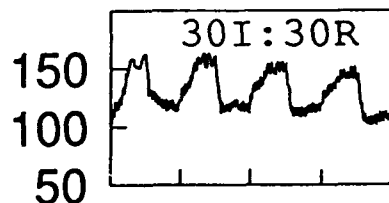
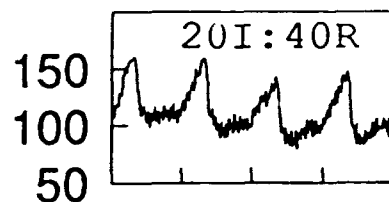
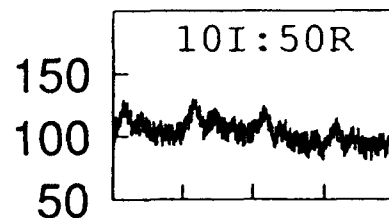
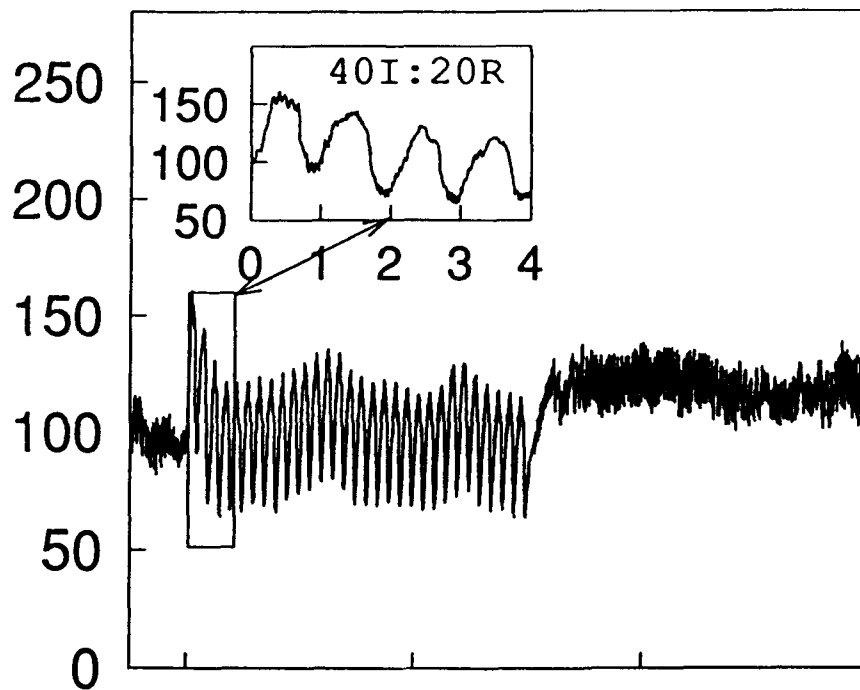
Figure 7. Lactate spectra acquired with one minute time resolution, during the initial control period (first spectra), immediately following thirty episodes of 40I:20R ischemia:reperfusion and finally subsequent to 30 minutes of unperturbed reflow. The observed resonances are: lactate 1.33 ppm; N-acetyl aspartate 2.02 ppm; glutamate, glutamine, aspartate and N-acetyl aspartate (2.1-2.8 ppm); creatine + phosphocreatine 3.03 ppm and choline 3.18 ppm. Note the marked increase in lactate following the ischemia:reflow cycles and the similarity in the magnitude of the 1.33 ppm control and reflow peaks.

Figure 8. Illustrated are the time course of Lactate and pH change, and the inter-relationship between them. Lactate values are one minute averages \pm SD while pH measurements are four minute running averages \pm SD. Lactate reaches steady state levels in the 10I:50R and 20I:40R animals following 10 to 12 cycles of ischemia:reflow. Although ischemic:reflow plateau levels of lactate are not attained in the 30I:30R and 40I:20R animals, the kinetics of lactate accumulation and pH loss in all animals are similar. Lactate clearance and pH recovery during the subsequent reflow period was complete. The ischemic pH and lactate data correlated significantly, however, the data was found to segregate according to duration of reflow time with decreased pH being associated with decreased reflow time at identical lactate levels.

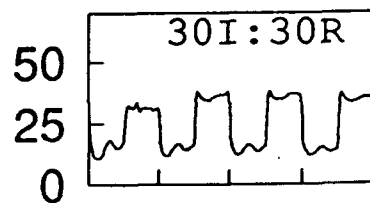
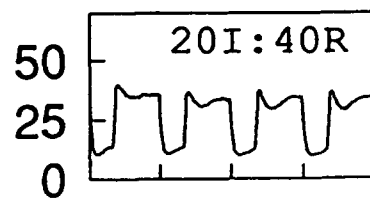
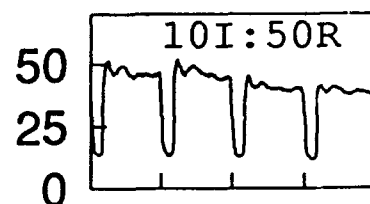
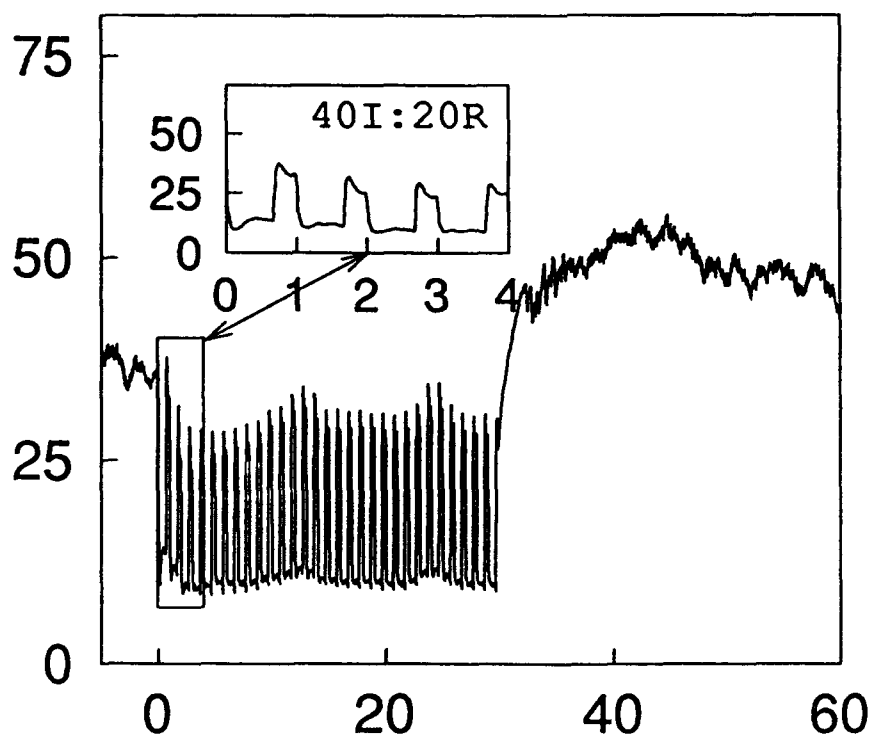
Figure 9. Correlations between EEG activity and measurements of P_i , pH and PCr are shown. Discrete symbols permit identification of individual ischemic:reflow groups as identified in the legend. Values of correlation coefficients reflect exponential fits for P_i vs EEG activity, while the correlation coefficients between pH and PCr and EEG activity reflect linear fits.



MABP (mmHg)

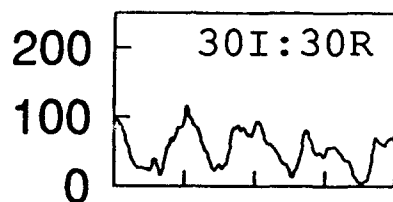
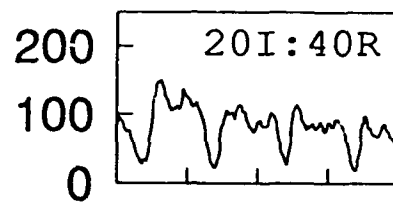
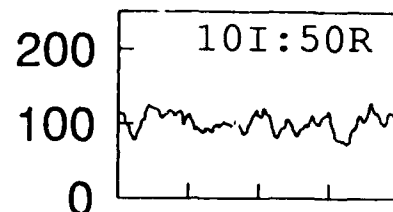
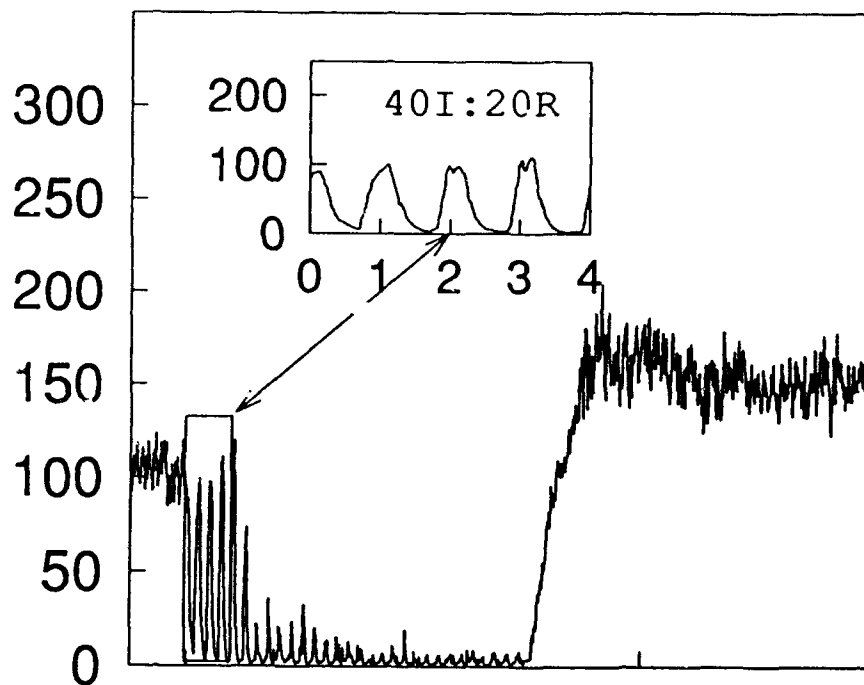


CSP (mmHg)

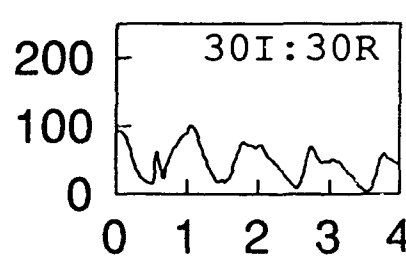
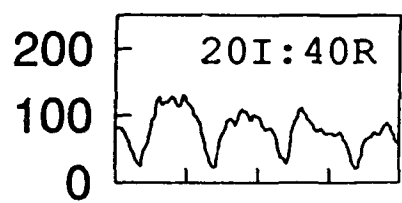
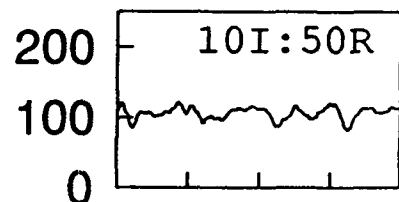
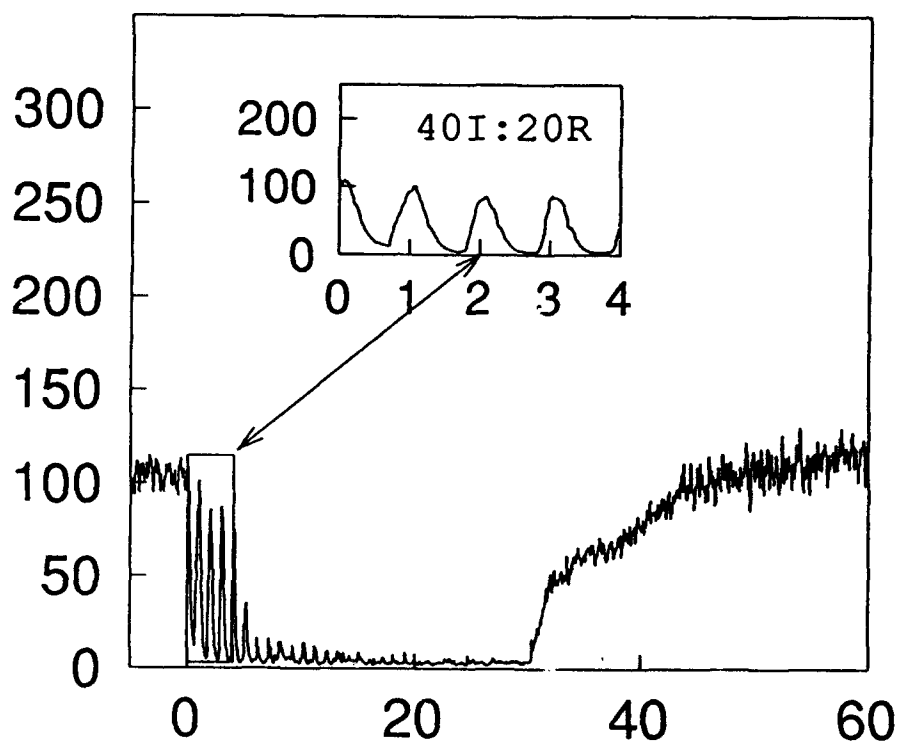


Time(minutes)

2-5 Hz Activity(%)

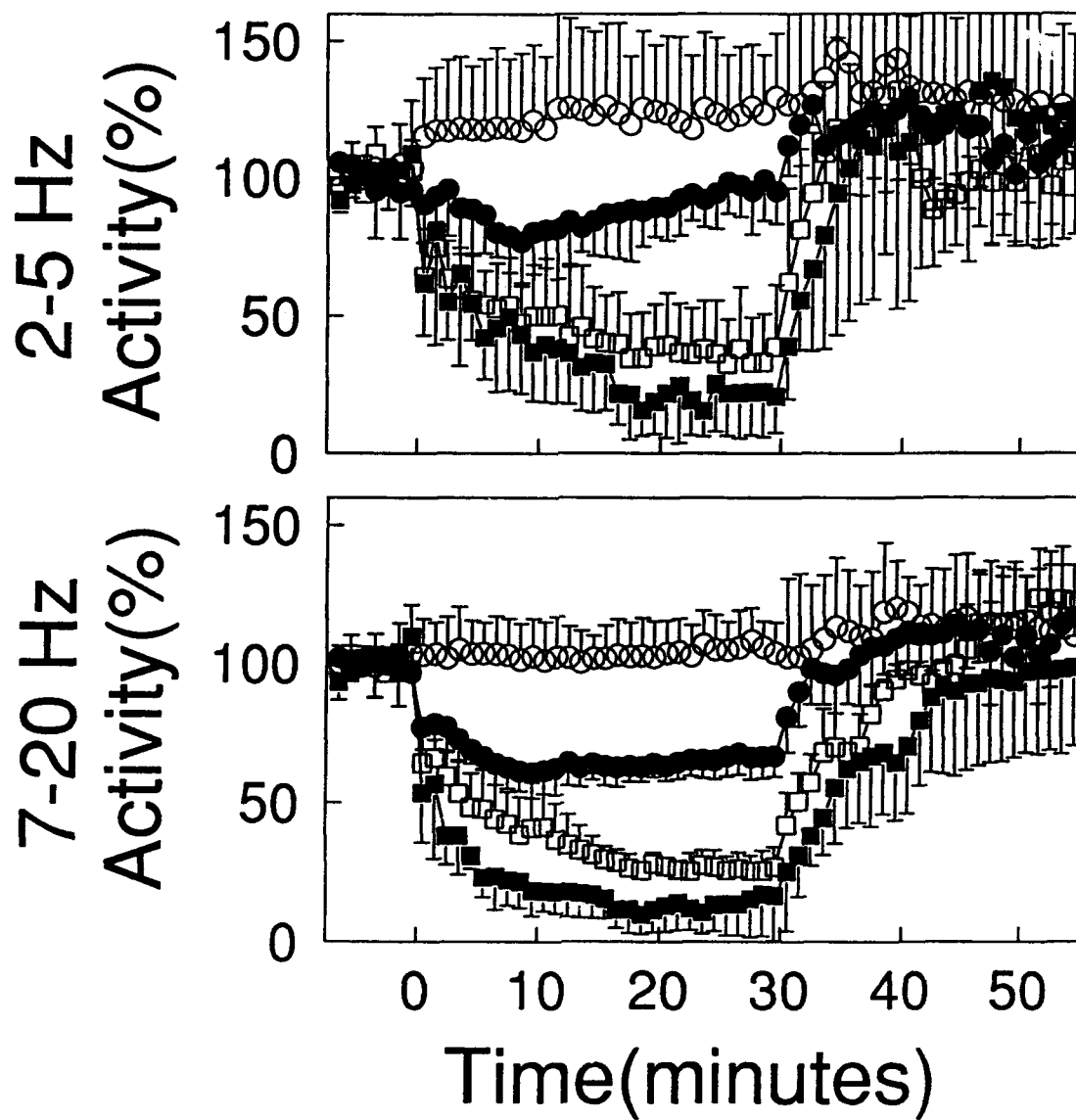


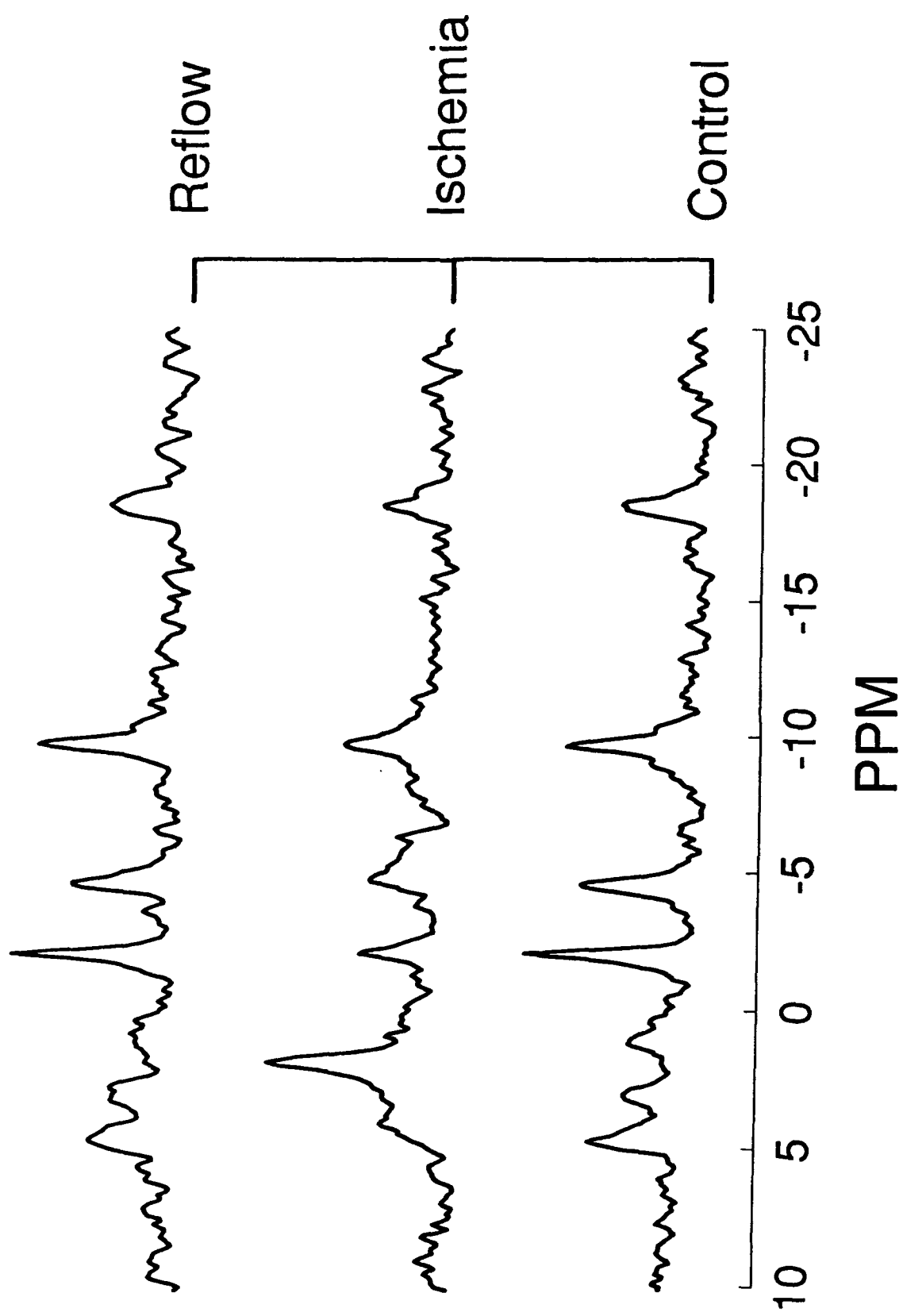
7-20 Hz Activity(%)



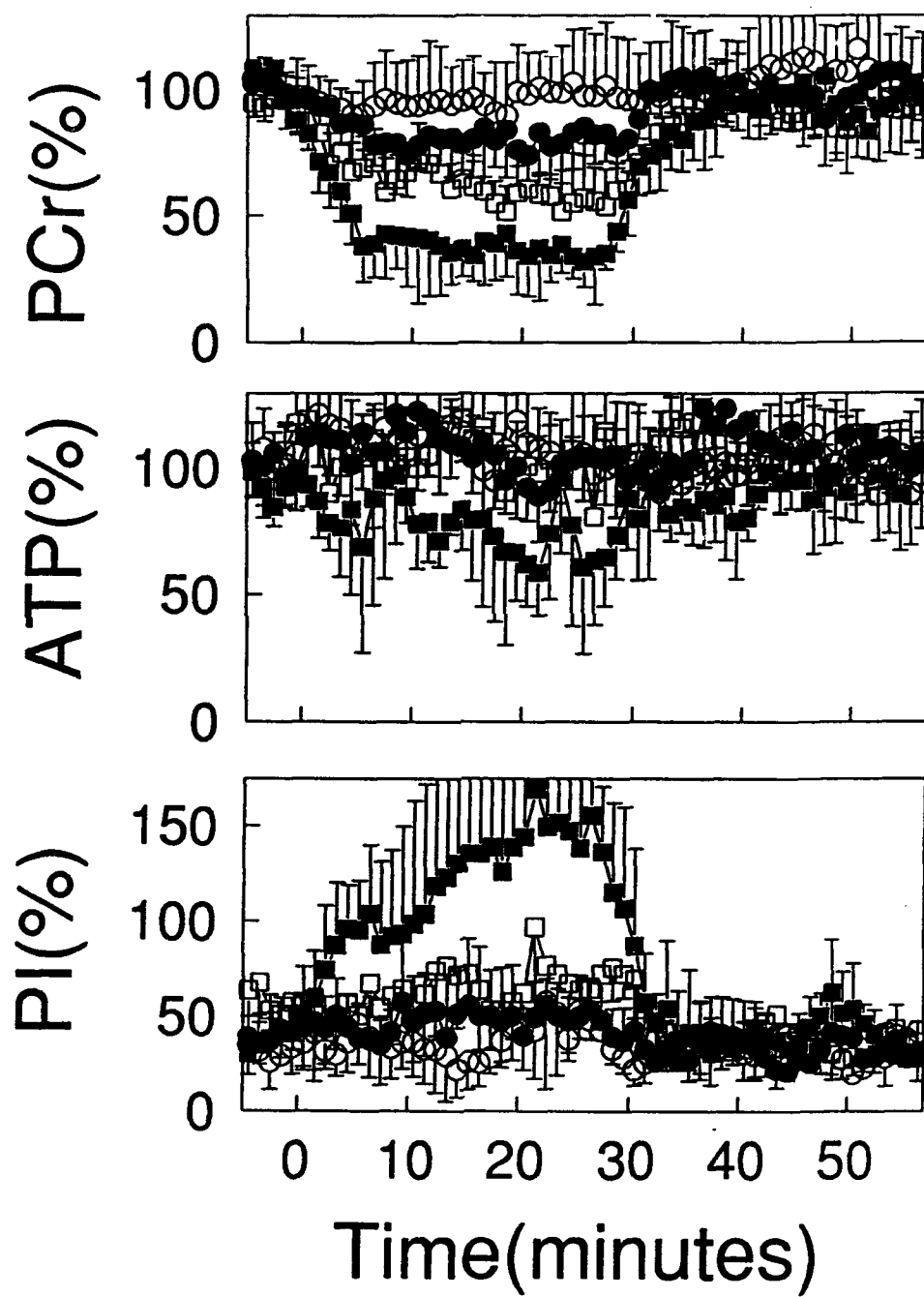
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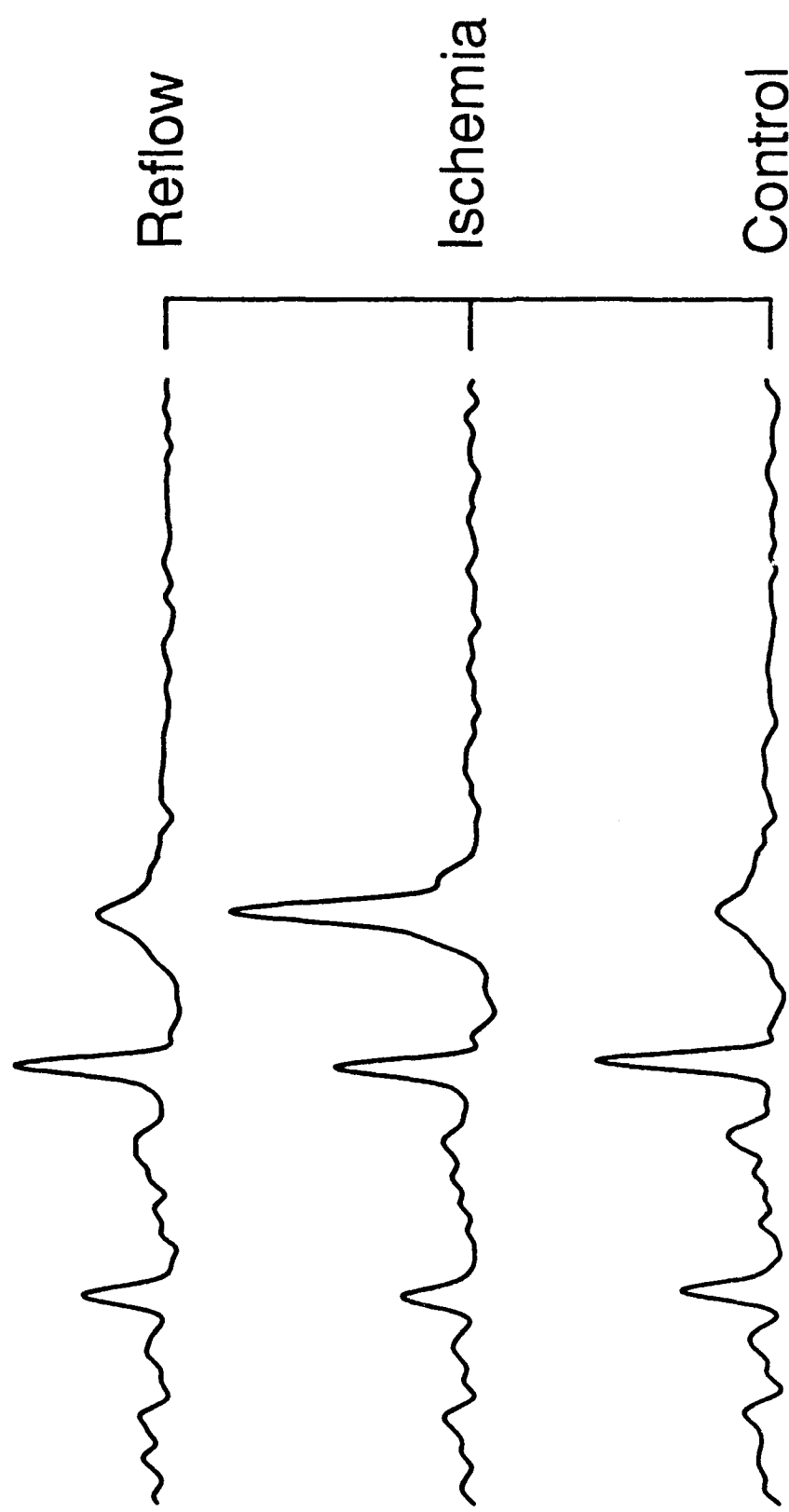
○ 10I:50R ● 20I:40R □ 30I:30R ■ 40I:20R



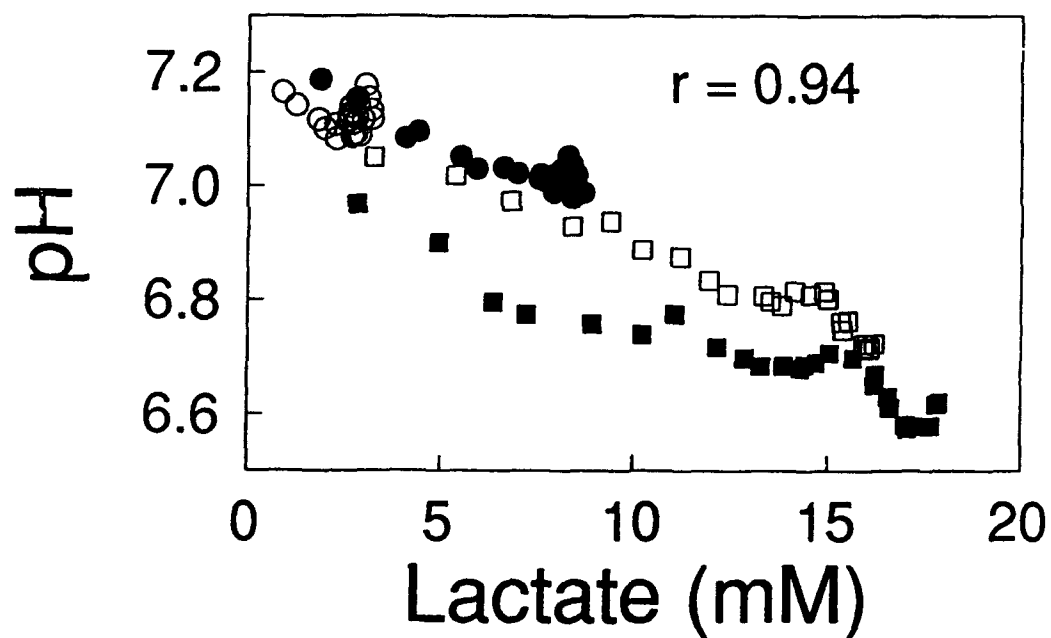
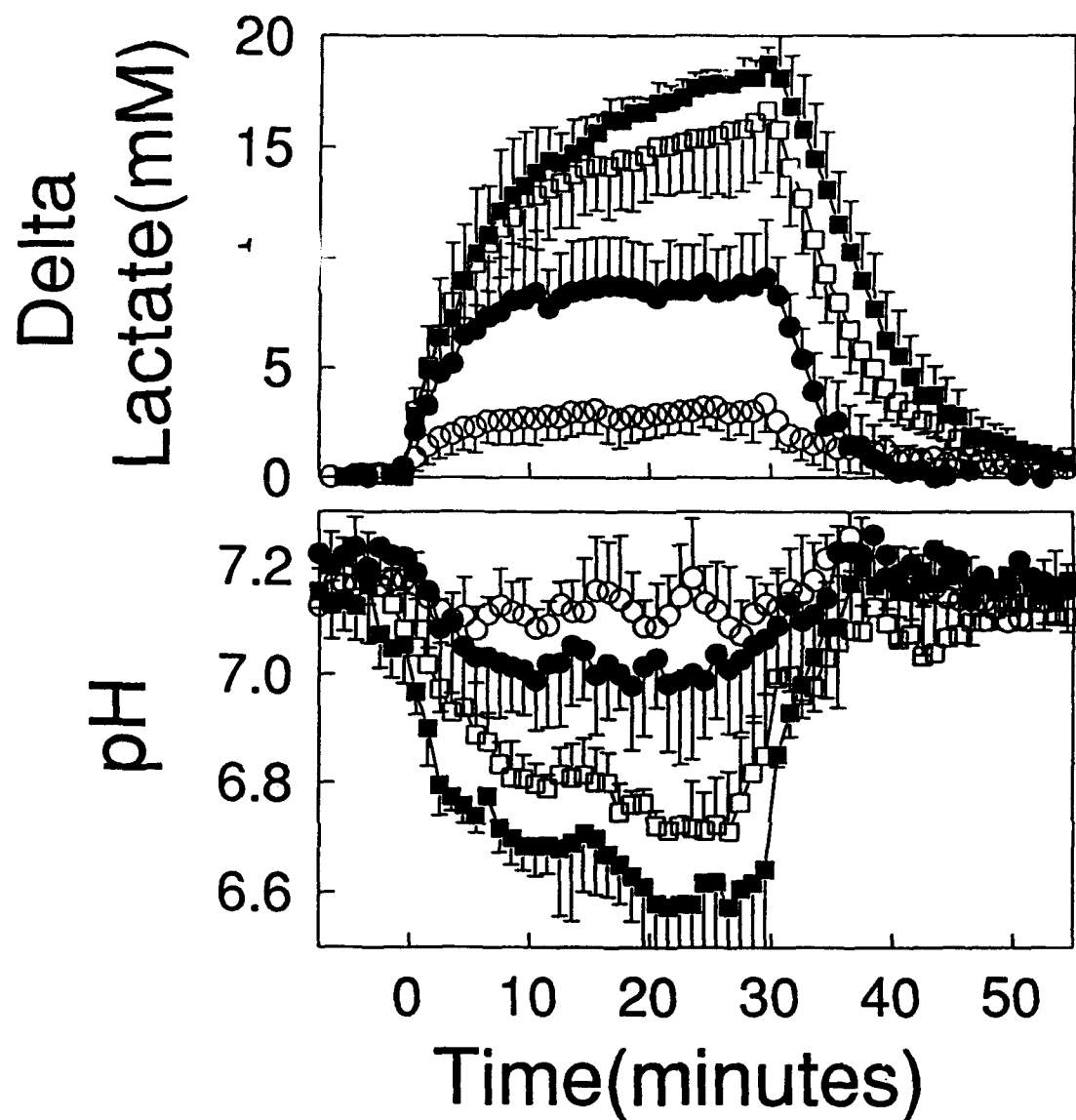


○ 10I:50R ● 20I:40R □ 30I:30R ■ 40I:20R



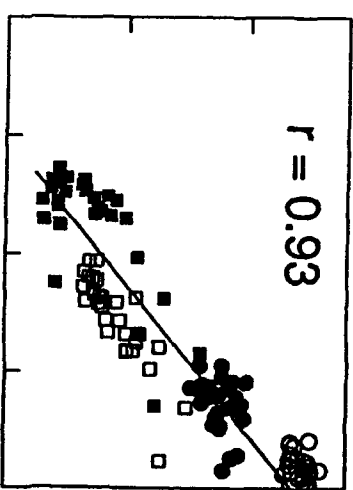
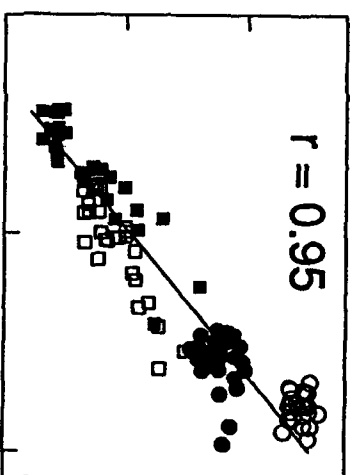
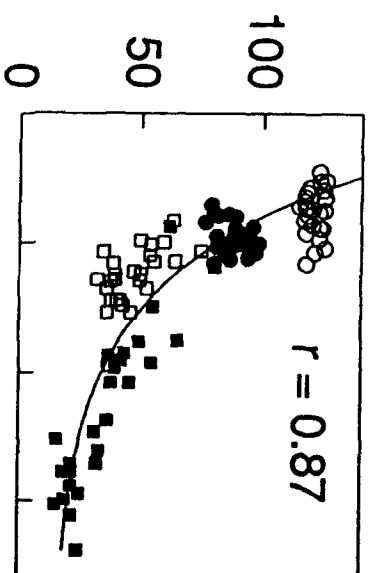


○ 10I:50R ● 20I:40R □ 30I:30R ■ 40I:20R

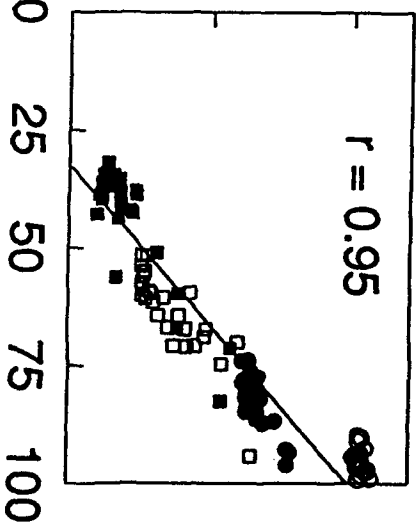
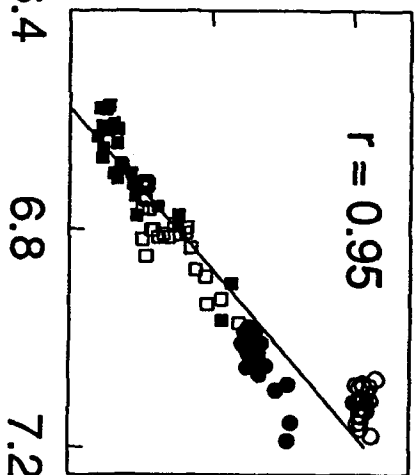
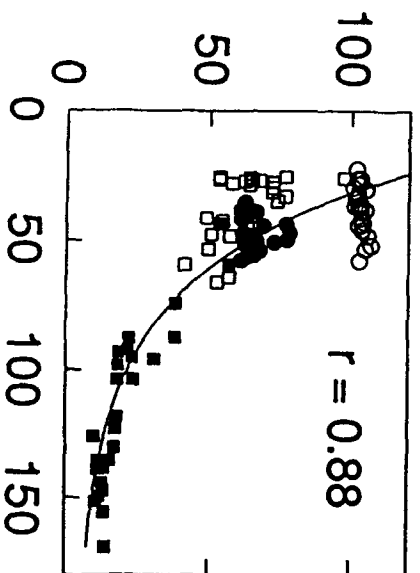


○ 10I:50R • 20I:40R ◻ 30I:30R ▪ 40I:20R

2-5 Hz
Activity (%)



7-20 Hz
Activity (%)



Pi (%)

pH

PCr (%)

Simulating Lactate Accumulation During Repeated Brief Cerebral Ischemia

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Introduction:

Pilots of high performance aircraft are subject to transient loss of consciousness due to sudden high gravitational stress. This is due to cerebral ischemia resulting from failure of blood delivery to the brain due to the severe gravitational stress. We have developed an animal model whereby the problem of multiple Gz-induced blackouts can be investigated. The objective was to determine if the time course of lactate accumulation could be predicted from measurements of glycolytic rate made prior to the multiple occlusion period and measurements of terminal washout rates.

Methods:

A) Experimental: Six rats were anesthetized with 70% N₂O, 29% O₂, and 1% Halothane. The rats were paralyzed with tubocurarine chloride and mechanically ventilated. Rats were prepared by ligation of both subclavian and external carotid arteries. The right carotid was cannulated distally for monitoring of carotid stump pressure (reflecting cerebral perfusion pressure at the Circle of Willis). Systemic arterial pressure, rectal and mouth temperatures were monitored and mouth temperature was controlled at 37°C. An occlusive cuff was placed around the remaining left carotid (fig.1). Prior to and following the multi-occlusion experiment rates of lactate generation were determined during one minute test occlusions. Plasma glucose, hematocrit and arterial blood gases were determined immediately prior to the first episode of ischemia. Thirty 30 second episodes of global brain ischemia were made by temporary inflation of the carotid balloon while ¹H spectra were acquired at 10 second intervals, 8 acquisitions using a semi-selective spin echo sequence, T_e=136ms, T_r=1.25s, with a 4.7T Bruker Biospec system. Each occlusive period was followed by 30 seconds of reflow.

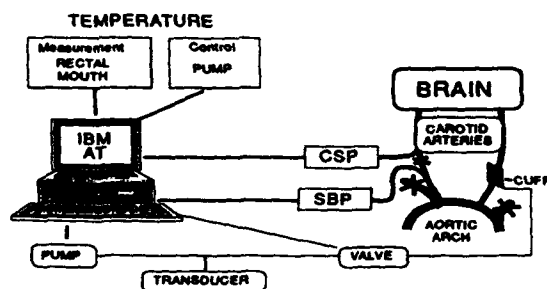


Figure 1. Block diagram of the components in the automatic occluder system. The computer monitors carotid, systemic blood and occluder system pressures. Computer monitoring and control of mouth and rectal temperatures was also implemented.

B) Simulation: The simulation is designed to describe the principal components that contribute to determine the shape of lactate accumulation curves found experimentally in our multi-occlusion model. The simulation is based entirely on experimentally determined values, therefore, no fitting parameters are applied in the simulation. Prior to the multi-occlusion experiment depicted in figure 2 (results), the animal was subjected to a one minute occlusion. A least squares linear fit to the measured change in lactate gave us an estimated 4.58 ± 0.26 mM/min rate for lactate generation during ischemia. To obtain a best estimate of lactate clearance rates, we fit a monoexponential to the lactate washout curve following the multiple occlusions. The rate of lactate loss could be defined as follows:

$$\text{Lactate Loss rate (mM/min)} = -0.0878/\text{min} \times \text{Lactate (mM)}$$

The experimental results were then simulated for the thirty cycles of occlusion-reperfusion by generating lactate linearly during each occlusion and decreasing lactate exponentially during each reperfusion period.

Results:

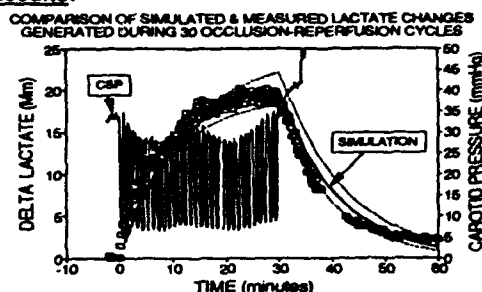


Figure 2. The illustration combines experimentally measured values of the carotid stump pressure (CSP) and delta lactate plotted as boxes. The simulation of lactate change is given by the solid line. The dashed lines represent simulations reflecting \pm SEM for the exponential fit to the washout data.

The exponential rate constants for washout of lactate ranged from .089 to .193 min⁻¹ whereas ischemic lactate production rates ranged from 4.0 to 7.2 mM/min, with lower rates corresponding to higher perfusion pressures during occlusion.

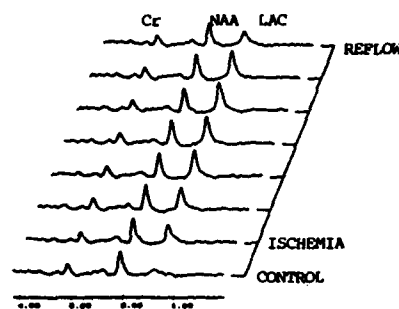


Figure 3. Selected, 8 acquisition (10sec) ¹H spectra collected during: the control period, at 5 minute intervals during ischemia and after 8 minutes of reflow. The labeled resonances are Cr, total creatine, NAA, N-acetylaspartate and LAC lactate.

Conclusions:

We believe that the occlusive model functions as follows: With each occlusion lactate is generated linearly at the maximum catalytic rate of the glycolytic pathway. Glucose is not exhausted during the short occlusive process, and is subsequently replenished in the short reperfusion periods following occlusion therefore, absolute glucose brain or plasma levels do not affect lactate levels. Lactate concentrations reach plateau levels because lactate clearance increases exponentially until the clearance rate matches lactate generation rates. It has been suggested (1) that the carrier mediated transport of lactate may be the limiting factor responsible for the observed rapid build-up of lactate during hypoxemia and the slow removal of lactate in post-hypoxic recovery (2).

References:

1. Pardridge W.: *J Neurochem* 28:5-12, 1977
2. Drewes LR.: *J Biol Chem* 248:2489-2496, 1973.

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